From the Unit of Infectious Diseases
DEPARTMENT OF MEDICINE HUDDINGE

Karolinska Institutet, Stockholm, Sweden

ASSESSMENT OF PATIENTS WITH LATE DIAGNOSIS AND MISSED OPPORTUNITIES IN THE SWEDISH HIV-1 EPIDEMIC

Johanna Brännström

Karolinska Institutet

Stockholm 2016

Published by Karolinska Institutet.
Printed by E-Print AB

ISBN 978-91-7676-196-0

Assessment of patients with late diagnosis and missed opportunities in the Swedish HIV-1 epidemic

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Johanna Brännström
MD

Principal Supervisor:
Professor Anders Sönnerborg
Karolinska Institutet
Department of Medicine, Huddinge
Unit of Infectious Diseases

Co-supervisor(s):
Dr. Veronica Svedhem Johansson
Karolinska Institutet
Department of Medicine, Huddinge
Unit of Infectious Diseases

Dr. Gaetano Marrone
Karolinska University Hospital
Department of Infectious Diseases

Opponent:
Dr. Matti Ristola
University of Helsinki
Department of Clinical Medicine

Examination Board:
Associate professor Hans Norrgren
University of Lund
Department of Clinical Science
Division of Infection Medicine

Associate professor Jens Boman
University of Umeå
Department of Clinical Microbiology

Associate professor Carl-Johan Treutiger
Karolinska Institutet
Department of Medicine, Huddinge
Centre of Infectious Medicine

To my family

It is bad enough that people are dying from AIDS,
but no one should die of ignorance.

(Elisabeth Taylor, 1986)

ABSTRACT

Discovered in 1983, with a possibility to diagnose since 1985, and with efficient treatment existing now for two decades, HIV-1 still ranks among the top ten causes of death globally. Approximately 40 million people are living with HIV-1 worldwide; almost half still not diagnosed. In Europe one third are estimated to be unaware of their infection and half are diagnosed late, with consequences in terms of increased morbidity, mortality, risk of onward transmissions and higher health care costs. The aim of this thesis was to assess the extent of late diagnosis of HIV-1 infection in Sweden and to analyse whom gets diagnosed late and why.

For **Paper I** we conducted a retrospective study of all patients diagnosed with AIDS (n=487), reported to the Swedish Centre of Infectious Disease Control (SMI) 1996-2002, concluding that the patients diagnosed late (here defined as simultaneous HIV/AIDS) represented an increasing proportion of patients with AIDS in Sweden. Migrants, persons infected heterosexually and persons aged over 40; all had a higher probability of a late diagnosis.

For **Paper II** we conducted a cross-sectional national cohort-study including all newly HIV-1 diagnosed patients at 12 Swedish clinics. Data were collected from the National quality register InfCare HIV (n=575) and additional questionnaires (n=409) from the clinics. 58% were Late Presenters (LP), presenting for care with CD4+ T-cells < 350/mm3 +/- AIDS. Age (with increasing odds by increasing age) or being a migrant had a distinct association with being a LP. Half of the migrants had lived in Sweden for > 1 year at diagnosis and two thirds had a missed opportunity at immigration. However, if born abroad, but reported to be infected in Sweden, there was no difference in LP compared to the Swedish born. One quarter of all patients had missed opportunities within Swedish healthcare, presenting with HIV- and/or AIDS-associated symptoms, without an offer of HIV-testing. 16% had a history of self-neglected symptoms.

In **Paper III** we further analysed the missed presentations at seeking healthcare, the HIV- and AIDS- associated symptoms neglected by the patients and also assessed the initiator of the HIV-test. Migrants were less likely both to neglect their symptoms and to be missed at health care compared to individuals born in Sweden. Also men who have sex with men (MSM) were less likely to neglect their symptoms compared to those with a heterosexually acquired infection. Patients with a history of drug use, a previous negative test (mainly MSM) and those infected abroad were more likely to take the initiative to test, whereas the opposite held for patients >50 years and those previously missed at presentation.

The predominance of migrants in Papers II-III, and results indicating that the number of domestic infections might be underestimated in this group, made us want to investigate this further. In **Paper IV** we applied a CD4+ T-cell decline trajectory model to a subsample of the Swedish migrant cohort (n= 1244) to compare estimates of country of HIV acquisition with the clinical reports. The model estimated that 17% had acquired the HIV infection after immigration, whereas the doctor's estimate was 11%. Phylogenetic analysis was performed in discordant patients to explore whether this would favour the model or the doctor's estimate. A higher concordance was found with the CD4 model estimates than with the clinical reports (30% vs. 17%).

In summary my thesis shows a high proportion of late HIV-1 diagnosis in Sweden, but also emphasizes that there are several opportunities to improve this. Activities to increase societal awareness, continuous promotion and normalization of the HIV-test, education of health care professionals including further implementation of indicator-guided testing and an extended testing and primary prevention aimed at migrants are all important steps forward.

LIST OF SCIENTIFIC PAPERS

I. Brännström J, Åkerlund B, Arneborn M, Blaxhult A, Giesecke J. **Patients unaware of their HIV infection until AIDS diagnosis in Sweden 1996-2002--a remaining problem in the highly active antiretroviral therapy era.** *Int J STD AIDS, 2005; 16: 702-706.*

II. Brännström J, Svedhem Johansson V, Marrone G, Wendahl S, Yilmaz A, Blaxhult A, Sönnerborg A. **Deficiencies in the health care system contribute to a high rate of late HIV diagnosis in Sweden.** *HIV Medicine; 2015; doi: 10.1111/hiv.12321.*

III. Brännström J, Svedhem Johansson V, Marrone G, Andersson Ö, Azimi F, Blaxhult A, Sönnerborg A. **Symptomatic patients without epidemiological indicators of HIV are at higher risk of missed diagnosis: a multi-centre cross sectional study**. *Submitted.*

IV. Brännström J, Sönnerborg A, Svedhem Johansson V, Neogi U, Marrone G. **Evaluation of a CD4+ T-cell decline trajectory model in the Swedish migrant population suggests a higher rate of HIV-1 acquisition post immigration.** *In Manuscript.*

ADDITIONAL RELEVANT PUBLICATIONS

I. Mocroft A, Lundgren J, Antinori A, d'Arminio Monforte A, **Brännström J**, Bonnet F, Brockmeyer N, Casabona J , Castagna A , Costagliola D, De Wit S, Fätkenheuer G, Furrer H , Jadand C, Johnson A, Lazanas M, Leport C, Moreno S, Mussini C, Obel N, Post F, Reiss P, Sabin A, Skaletz-Rorowski A, Suarez-Loano I, Torti C, Warszawski J, Wittkop L, Zangerle R, Chene G, Raben D, Kirk O. Collaboration of Observational, H. I. V. Epidemiological Research Europe study in EuroCoord. **Late presentation for HIV care across Europe: Update from the Collaboration of Observational HIV Epidemiological Research Europe (COHERE) Study , 2010 to 2013.** *Euro Surveill, 2015; 20.*

II. Wiklander M, **Brännström J**, Svedhem V, Eriksson L E. **Development and psychometric testing of a barriers to HIV testing scale among individuals with HIV infection in Sweden; The Barriers to HIV Testing Scale - Karolinska Version.** *Health Qual Life Outcomes, 2015; 13: 185.*

CONTENTS

LIST OF ABBREVIATIONS

AIDS	Acquired Immune Deficiency Syndrome
ART	Antiretroviral therapy
CDC	Centre for Disease Control and Prevention
CD4	Cluster of Differentiation 4
CI	Confidence Interval
COHERE	The Collaboration of Observational HIV Epidemiological Research Europe
EEA	European Economic Area
ECDC	European Centre for Disease Prevention and Control
EFTA	European Free Trade Association
ESR	Erythrocyte Sedimentation Rate
EU	European Union
GP	General Practitioner
INSTI	Integrase Inhibitor
HAART	Highly Active Antiretroviral Therapy
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HSV	Herpes Simplex Virus
HTLV-III	Human T-Lymphocyte Virus III
Lgll	Lymphnodes
LP	Late Presenter of HIV-infection
LPAH	Late Presenter with Advanced HIV
LPnAH	Late Presenter non Advanced
MSM	Men who have Sex with Men
MTCT	Mother To Child Transmission
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitor
nLP	Non-Late Presenter
NRTI	Nucleoside/Nucleotide Inhibitor
OR	Odds Ratio
PCP	Pneumocystis Pneumonia
PHI	Primary HIV Infection
PI	Protease Inhibitor
PLWH	People Living with HIV
PML	Progressive Multifocal Leukoencephalopathy
PWID	People Who Inject Drugs
SSA	Sub-Saharan Africa
STI	Sexually Transmitted Infection
TasP	Treatment as Prevention
UK	United Kingdom
UNAIDS	The Joint United Nations Programme on HIV and AIDS
US	United States of America
VL	Viral Load
VZV	Varicella Zoster Virus
WHO	World Health Organization

1 PREFACE

Why HIV became my main field of interest & the background of this thesis:

Growing up, being an adolescent, in the eighties and meeting information about HIV and AIDS almost anywhere made a deep impression on me. Lucky to have the most inspiring and engaged biology teacher, daring to confront the problem, I was early involved in vivid discussions from basic virology to the social stigma. A few years later, in parallel with medical studies in the early 90´s, meeting the epidemic "real life" while going clubbing in the gay Stockholm, dealing with the complex mix of party and fear, made imprints for life. Suddenly seeing the big miracle with the introduction of protease-inhibitors happening to be a medical student at the clinic of Infectious diseases at Danderyd hospital in 1996 was amazing. Everything was looking bright, but then, as a newly fledged specialist in Infectious diseases several years later, deciding to specialize in HIV, I still met very sick patients… My first strong impression was a woman my own age having been ill for years with skin problems, fungal infections, anaemia and weight loss finally sent into the Clinic of Infectious Diseases for recurrent pneumonias. In spite of several contacts with the Swedish health care system no one had had a thought of HIV.

There were still many challenges…

2 INTRODUCTION

2.1 GENERAL ASPECTS OF LATE HIV-1 DIAGNOSIS

Despite advances in prevention, diagnostics and treatment, the human immunodeficiency virus (HIV) is still one of the leading deaths causes worldwide and remains one of the most important communicable diseases in Europe. Estimates show that as many as one third of HIV-1 positive individuals living in the European region could be unaware of their diagnosis and about half are diagnosed late [1, 2] with consequences in terms of increased morbidity, mortality [3], an increased risk of onward transmission [4] and consequently also higher health care costs [5].

There is extensive research on vaccination and cure on-going [6], but still, in the absence of this, early diagnosis and treatment is one of the most important approaches to reduce HIV-related morbidity and to control the HIV epidemic.

The reasons for late diagnosis of HIV-1 are many and diverse. The overall aim of this thesis was to assess the situation in Sweden by quantifying the proportion diagnosed late, identifying risk indicators associated with a late diagnosis and to identify opportunities for an earlier care of our patients.

As a HIV clinician I have had a special focus on the clinical manifestations of the disease and how I could contribute to enhance earlier diagnosis by assessing the doctors' and patients' delay, respectively. Thus a substantial part of the background also deals with the different phases of infection and what clinical signs and laboratory findings it is important to pay attention to. The large proportion of migrants among our patients, and a general interest in public health and communicable disease control, also made me raise my eyes and incorporate missed opportunities on the societal level by assessing the diagnosis of HIV-1 infection in migrants from high endemic countries.

2.2 HIV HISTORY

The first official report on what was later to be known as HIV/AIDS, was published in 1981 [7], describing 5 cases of unusual lung infection, Pneumocystis carinii (later known as Pneumocystis jirovecii) pneumonia (PCP) in homosexual men, in Los Angeles, USA. Shortly similar cases, including a cluster of Kaposi's sarcoma among homosexual males in other cities, were reported. Among these were also diagnoses of candida infections, severe herpes infections, PCP, cryptococcal meningitis and cerebral toxoplasmosis, all signs of an impaired immune system [8-11].

Speculation and theories of the cause of the disease were many and imaginative, not seldom coloured by prejudice. However, soon it became evident that this disease did not only affect homosexuals but also haemophiliacs, people who inject drugs (PWID), males/females from Haiti and partners to these as well as children born by women in these groups [12]. In the mid-eighties the epidemic in Africa, where HIV have its origin, was observed [13]; HIV-1

crossing the art barrier and pass from chimpanzee to human approximately in the 1920/30'ies [14].

In 1983, the same year as the first Swedish patient with AIDS was identified at Roslagstull's hospital, a human retrovirus was isolated from lymph nodes by the Montagnier/Barré-Sinoussi group and confirmed by Robert Gallo [15, 16]. The virus, initially called LAV (Lymphadenopathy Associated virus)/HTLV-III (Human T-Lymphocyte Virus type III), but later renamed as HIV-1, was identified as the causative agent of AIDS.

The isolation of the virus lead to the first commercial blood test to detect HIV antibodies in the blood, ELISA [17, 18], which was licensed 1985. In parallel to the medical progress demands of testing in different groups evoke resulting in severe consequences for those turning out to be HIV positive, contributing to an already started stigmatization.

In 1987 the first anti-retroviral drug, zidovudine (zdv), was introduced [19], prolonging life, but in retrospect causing problems with viral resistance. In June 1995 the first Protease Inhibitor was approved [20] and thereby starting a new era with combination of medications, so called, Highly Active Anti-Retroviral Therapy (HAART) or cART (c=combination), rapidly reducing AIDS morbidity and mortality rates in those with access to treatment. The following year, 1996, PCR methods to measure HIV RNA were introduced and high viral loads were seen to be associated with a poorer prognosis [21].

From 1996 there is the ability to diagnose, efficiently treat and monitor a HIV infected patient. However, people still die of AIDS and the HIV related stigma continues…

2.3 THE VIRUS

HIV is a retrovirus within the genus Lentiviridae (lenti=slow) of the Retroviridae family [22]. The virion is composed of two copies of a single-stranded RNA and set of viral proteins, including the viral enzymes, and surrounded by a lipid bilayer membrane, originally derived from the infected host cell [23] (*Figure 1*).

The viral genome is composed of nine genes, out of which the three structural genes; gag, pol and env, are the most important. Gag encodes for the major structural proteins (e.g. the matrix protein (p17) and the capsid protein (p24)). The pol genes encode for the viral enzymes essential for the reverse transcription of RNA to DNA (reverse transcriptase, p64), integration of HIV DNA into the human genome (integrase, p32) and cleavage of the HIV proteins (protease, p10). Env encodes for the envelope glycoproteins (gp41 and 120), crucial for the virus ability to infect the human target cells, by attaching to their CD4 receptors [23, 24] (*Figure 1*).

The viral enzymes are each important prime therapeutic targets for ART and sequencing of the pol gene is recommended to monitor drug resistance. These sequences can also be used in constructing phylogenetic trees, a technique used in paper IV.

There are two main types; HIV-1 and HIV-2, and the prior is what is focused on in this thesis.

The HIV-1 strains can be classified into four groups, originating from separate introduction of simian virus to humans. The "major" group M, constitutes > 90% of all HIV-1 infections worldwide. There are also the "outlier" group O and the more rare groups "novel", N, and P,

all mainly restricted to West and Central Africa. The group M is further subdivided into nine subtypes or clades; A-D, F-H, J and K [25]. Additionally there are mixtures of these; circulating recombinant forms (CRFs) and unique recombinant forms (URFs). HIV-1 subtype C was first discovered by Professor Sönnerborg's research team in 1988 [26] and is now the most common and constitute half of all infections worldwide [27]. In resource rich countries the subtype B historically have been the predominant, but other sub-types are becoming more frequent as a result of travels and migration. In Sweden subtype B today constitutes less the 50% and recombinant forms are increasing among newly diagnosed [28] showing that we are part of the global epidemic.

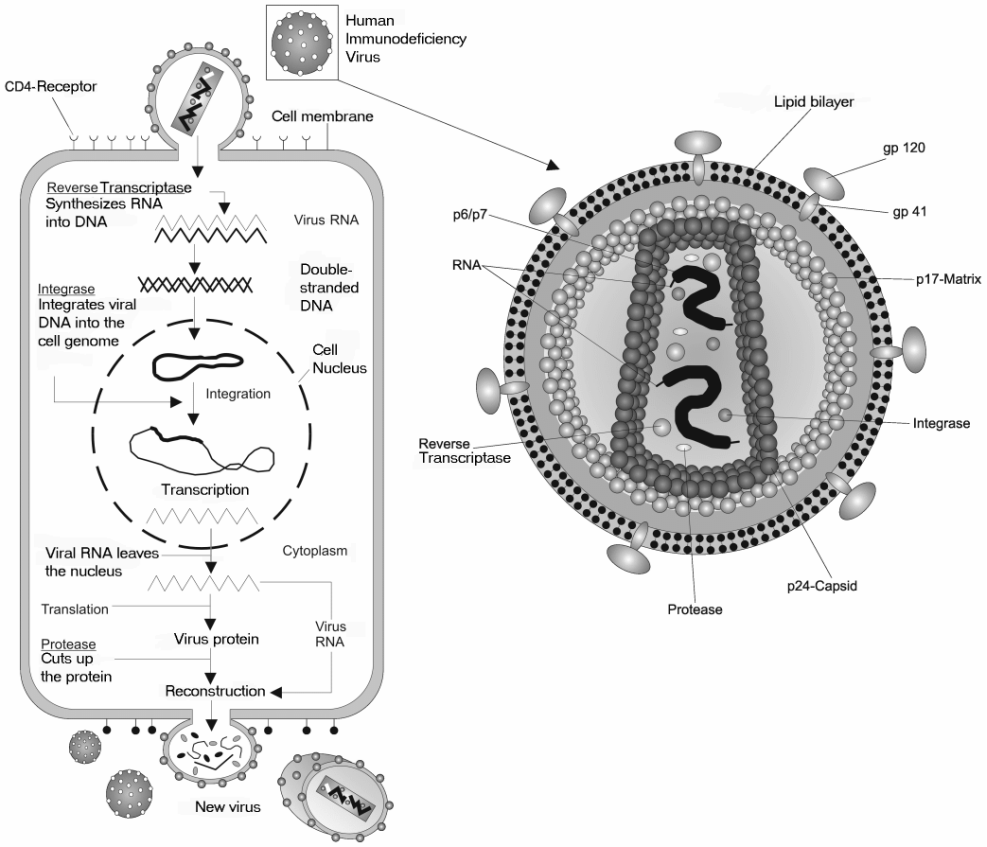

Figure 1. *The structure and life cycle of HIV . Source: www.study.com/academy*

2.4 THE HOST AND THE T-HELPER CELL

By attaching to the CD4 receptor and the chemokine co-receptor CCR5, or in some cases CXCR4, the target cells of HIV are entered.

The most important cells affected by HIV are the CD4+ T-lymphocytes (the "T-helper cells"), which have a central role in our immune system by directing a variety functions.

By destroying and deregulating the CD4+ T-lymphocytes HIV also causes immunologic dysfunction of CD8+ T-lymphocytes, B-lymphocytes, natural killer (NK) cells, and non-lymphoid cells through mechanisms including increased cell turnover, immune activation,

differentiation, and homeostatic responses [29]. Together, all these factors lead to severe qualitative changes ultimately affecting the overall immunological competence of the host.

Due to its central role, progression of the HIV disease can easily be measured and evaluated by the CD4+ T-lymphocyte cell count, which is widely used as an estimate of the global immune competence [30]. This can be expressed as the absolute count, as the ratio CD4/CD8 or as the percentage of all lymphocytes. The prior is what is normally being used, even though the ratio has proved to be a better and more stable predictor of the immune pathology associated with HIV infection [31]. In non HIV-infected adults/adolescents normal CD4+ T-cell counts ranges from 490–1340 cells/mm^3 (corresponding to 1.13-3.93 or 35-59%, according to *the Clinical Immunology and Transfusion Medicine, Karolinska University Hospital*.

2.5 THE NATURAL COURSE OF HIV-1 INFECTION

2.5.1 Transmission

HIV is present in all body fluids of an HIV infected individual including blood, semen, pre-seminal fluid, vaginal fluids, rectal fluids and breast milk and is usually divided into four main routes of transmission; heterosexual contact, men who have sex with men (MSM), intravenous drug use (IDU) and mother to child transmission (MTCT). The later occurs predominantly at birth and by breastfeeding. Rarely, these days (after the introduction of screening for HIV antibodies in donated blood), it may also be transmitted by blood-products and transfusions.

Transmission rates from an untreated individual are highest by parental exposure and historically, before blood donor screening, blood transfusion was associated with nearly a 100% risk [32]. The risk of MTCT varies from 15-45%, whether the mother is breastfeeding or not, whereas for people who inject drugs (PWID) the risk of transmission per contaminated injection is estimated to 0.6-0.8%. Sexual transmission risk at mucosal exposure are reported to range widely from 3.4 to less than 0.05% and vary according to the site of exposure; rectal mucosa > vaginal mucosa > oral mucosa [33, 34]. Local inflammation, particularly in the presence of ulcers, increases the risk, just as the amount of body fluid and the magnitude of the inoculum (i e the level of plasma HIV RNA) [35]. The risk of transmission is also influenced by the innate immunity of the host [36].

2.5.1 Primary infection

After transmission, the virus disseminates to lymphoid tissues and replicates aggressively. Persistent viral reservoirs, with integration of viral DNA, predominantly in memory T-cells, are established almost instantly and the chronic infection is a fact [37].

The initial high viral replication may manifest itself through the acute HIV syndrome within one to four weeks after infection (*Figure 2*). This, often flu- or mononucleosis- like illness, known as acute or primary HIV infection (PHI), may last from a few days up to a month. However, the symptoms of PHI may also be mild enough to go completely unnoticed.

In the absence of ART the viremia peaks at about three to four weeks post exposure [38]. This is usually associated with a pronounced depletion of susceptible CD4+ T-cells in the body, primarily in the Gut Associated Lymphoid Tissue (GALT), but also in the peripheral blood [29, 39]. In rare cases the immunosuppression may become so severe, already at this stage, that the patient may develop AIDS defining conditions (*see 2.5.4.1. below*).

Due to the high viral replication the risk of onward transmission is very high and the newly infected individuals may actually be the ones mainly driving the epidemic [40].

2.5.1.1 Possible symptoms of Primary HIV-1 Infection include:

- *Fever*
- *Headache*
- *Myalgia/arthralgia*
- *Lymphadenopathy*
- *Pharyngitis*
- *Skin rash*
- *Night sweats*
- *Diarrhea*
- *Oral or genital ulcers*
- *Meningitis/meningoencephalitis, in rare cases.*

2.5.1.2 Laboratory findings in Primary HIV-1 Infection:

- *Anaemia, leukopenia and/or thrombocytopenia is common*
- *Elevated liver enzymes (ASAT/ALAT)*
- *Normal to slightly increased Crp*
- *Elevated LD*

2.5.2 Chronic asymptomatic infection – "Clinical Latency"

In parallel with the high viremia an HIV-specific, but incomplete, immune response leads to the development of a "steady state" or viral set point, typically reached within a few months. The level of this "set point" is an important prognostic factor; the higher viral load at this stage the more rapid disease progression [21]. The CD4+ T-cells typically rebounds to higher levels after the "steady state" has been reached, but seldom to its previous levels.

The above is followed by an asymptomatic chronic infection, where there is a sustained lower viral replication and immune activation [29]. During this phase there is a progressive loss of CD4+ T-cells (*Figure 2*). The mechanisms behind this depletion of T-cells have for many years been uncertain. Recently however it has been shown that only a minority, 5%, of dying cells are productively infected and die by apoptosis, whereas the remaining die by pyroptosis, a pro-inflammatory programmed cell death, rather to compare with a "cell-suicide", by which the cell prevents itself from becoming infected [41].

During this "clinical latency" stage of the HIV infection the circulating levels of HIV-virus in the blood are normally low, in rare cases even undetectable. Apart from a persistent lymphadenopathy, in some individuals, the infection normally remains asymptomatic until the CD4+ T-cells decrease to < 350 cells/mm^3. The duration of this "latency" period may last

from a year or two to > 15 years in the so-called long term non progressors [42]. The rate of disease progression is dependent both on factors of the host (e.g. different HLA alleles and mutations of the CCR5 receptor [39]) and of the viral fitness, where the speed of the viral replication is directly correlated to a faster disease progression (*Figure 3*).

Importantly, during this asymptomatic stage the patient also is infectious to others.

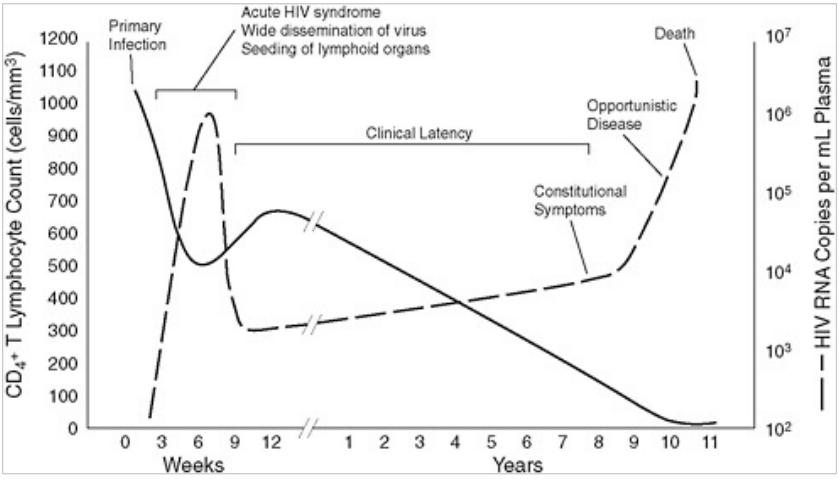

Figure 2. *Time-based progression of untreated HIV infection, demonstrated by CD4+ T-cell count and viral load. Adapted from Fauci and Pantaleo, 1993 [43].*

2.5.3 Chronic symptomatic infection

By time, as the CD4+ T-cells successively decrease and the immune system deteriorates, the "steady state" is eventually lost and the viral load increasing. In parallel with this the patient also becomes symptomatic (*Figure 2*).

Some of the early symptoms may be temporary and/or possible to treat and may be followed by more asymptomatic years and are thus important to recognize and pay attention to. Later constitutional symptoms develop.

2.5.3.1 Early symptoms in HIV-1 infection:

- *Varicella Zoster*
- *Seborrhoea*
- *Oral candida*
- *Hairy Leukoplakia*

2.5.3.2 Later, constitutional, symptoms in HIV-1 infection:

- *Fatigue*
- *Weight loss*
- *Fever, night sweets*
- *Diarrhea*

2.5.3.3 Laboratory findings in symptomatic chronic HIV-1 infection:

- *Anaemia, leukopenia and/or thrombocytopenia is common*
- *Elevated erythrocyte sedimentation rate (ESR)*

2.5.4 AIDS

Without ART the HIV-infection progresses to AIDS, which is the end-stage of the infection, in average after 8-10 years [44]. By the time AIDS develops the immune system is severely compromised with the risk of numerous opportunistic infections and development of certain cancers or other severe clinical manifestations, which most normally do not affect a healthy individual. Any of these infections is per se what is defining AIDS. The development of advanced symptoms are often corresponding to a CD4+ T-cell count of less than 200 cells/mm^3, a cut off that per se also are AIDS-defining in the American guidelines [45].

Without treatment AIDS leads to an inevitable death, but even with treatment the prognosis is much poorer than for someone having initiated treatment early [46].

2.5.4.1 AIDS-defining conditions:

- *Candidiasis of bronchi, trachea, or lungs*
- *Candidiasis of oesophagus*[†]
- *Cervical cancer, invasive*[§]
- *Coccidioidomycosis, disseminated or extra pulmonary*
- *Cryptococcosis, extra pulmonary*
- *Cryptosporidiosis, chronic intestinal (>1 month's duration)*
- *Cytomegalovirus disease (other than liver, spleen or nodes)*
- *Cytomegalovirus retinitis (with loss of vision)*[†]
- *Encephalopathy, HIV related*
- *Herpes simplex: chronic ulcers (>1 month's duration) or bronchitis, pneumonitis, or esophagitis*
- *Histoplasmosis, disseminated or extra pulmonary*
- *Isosporiasis, chronic intestinal (>1 month's duration)*
- *Kaposi sarcoma*[†]
- *Lymphoma, Burkitt*
- *Lymphoma, immunoblastic*
- *Lymphoma, primary, of brain*
- *Mycobacterium avium complex or Mycobacterium kansasii, disseminated or extra pulmonary*[†]
- *Mycobacterium tuberculosis of any site, pulmonary,*[†§] *disseminated,*[†] *or extra pulmonary*[†]
- *Mycobacterium, other species or unidentified species, disseminated*[†] *or extrapulmonary*[†]
- *Pneumocystis jirovecii pneumonia*[†]
- *Pneumonia, recurrent*[†§]
- *Progressive multifocal leukoencephalopathy*
- *Salmonella septicemia, recurrent*
- *Toxoplasmosis of brain, onset at age >1 month*[†]
- *Wasting syndrome attributed to HIV*

[†] Condition that might be diagnosed presumptively. [§] Only among adults and adolescents aged ≥13 years. (CDC. 1993 Revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. MMWR 1992;41)

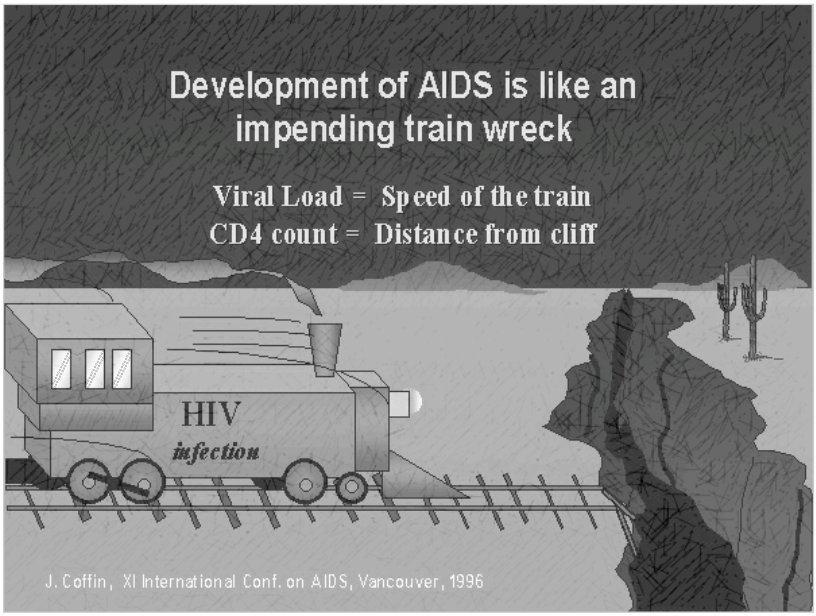

Figure 3. *HIV and viral dynamics, from J Coffin 11th International Conference on AIDS; 1996; Vancouver, Canada. Abstract Th.18.*

2.5.4.2 Symptoms of AIDS:

- *Night sweats*
- *Weight loss*
- *Unexplained fatigue*
- *Persistent fever*
- *Chronic diarrhoea*
- *Skin rashes*
- *Tongue lesions*
- *Cough*
- *Shortness of breath*
- *Headaches*
- *Blurred and distorted vision*

2.6 DIAGNOSING HIV

The diagnosis of HIV is primarily based on serology methods, detecting antibodies to the virus by an Enzyme Linked Immuno Assay (ELISA). Most commonly, in resource rich settings, a combination assay, detecting also the HIV p24 antigen, is being used. An infected person is most often reactive in this combination test within 2-3 weeks but a follow-up time of six weeks is recommended to exclude HIV infection [47]. If a test is reactive a second, more specific immunoassay (immunoblotting or Western blot) is used, both to confirm the positive result and to differ between HIV-1 and 2.

The test has a high sensitivity and specificity, >99%, and results are normally available within 24 hours. For even quicker results a rapid test (either on blood or saliva) may be used. Also this kind of test has a high accuracy, but have a poorer capacity to catch the earliest infections and, in case of a positive result, also require confirmation with conventional methods.

During a true symptomatic PHI the combo test is always positive, but if there is a high suspicion of a very early HIV infection a PCR test to detect the viral RNA can be considered. However the window when the combo test is negative and the PCR test is positive consists of a few days only shortly after transmission [47].

In all patients diagnosed with HIV an additional test on a second blood sample is always made before making the diagnosis definite. This is done just to make sure there has been no mix up of laboratory samples.

Despite excellent and also cheap diagnostics too few tests are offered…

2.7 HIV TREATMENT

From having been associated with an imminent death sentence, ART has transformed the HIV infection to a treatable chronic disease [48, 49].

Since the introduction of the first antiretroviral drug (ARV), zidovudine (zdv) in 1987 approximately 30 additional drugs, in 6 different classes, have been approved. From 1996, when the Protease inhibitors were introduced, it is possible, by combining ARVs with different mechanisms of action, to fully suppress the viral replication. By this not only further deterioration of the immune system is prevented; it also allows for improvement and recovery. The treatment is life long, but even though it cannot cure HIV, it allows for people living with HIV to have "healthy and productive lives" and a near to normal life span [50-53]. Furthermore, a well-treated patient has a negligible risk of transmitting the disease also making big implications in terms of prevention *(see 3.3.2)*.

HIV-1 treatment is normally given by combining 3 different ARVs, where the typical combination for many years have consisted of 2 Nucleoside/Nucleotide Reverse Transcriptase Inhibitors (NRTIs) in combination with a Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI), a boosted Protease Inhibitor (PI/r) or an Integrase Inhibitor (INSTI). Finally there are also Entry/Fusion Inhibitors, mainly used in patients with resistance to the other drugs. The last years the INSTI have started to dominate as the drug of choice in the newly diagnosed patients, still with 2 NRTIs as backbone, but NRTI sparing regimens are being investigated in order to minimize side effects. Studies of the "nuce-lite" regimen of lamivudine (3TC) and a boosted PI have recently shown non-inferiority compared to standard ART [54-56]. Similarly an on-going study, investigating dual-therapy with 3TC and INST (dolutegravir), presents promising interim-results [57].

Further development of ART drugs is on-going, not the least by finding new formulas of existing medications, to allow for fewer side effects and to make them easier to administer.

When to start ART has been debated since it was first introduced and the approach has changed from treating only the most immunocompromised with symptomatic disease, to treat also the asymptomatic according to different cut of levels of CD4+ T-cells of 200, 350 and 500 respectively. During 2015 the START [58] and TEMPRANO [59] studies presented evidence in favour of immediate treatment and ART are now recommended to everyone with HIV-1 disregarding the CD4+ T-cell count [60-62].

To fully benefit from ART early diagnosis and initiation is needed…

2.8 HIV VACCINATION AND CURE

Even though modern ART are efficient, easy to administer and associated with minimal side effects HIV is still, by many, considered to be associated with several comorbidities due to residual viral replication and/or chronic inflammation [63-66]. There is also the obstacle of taking drugs, the worries of onward transmission and last but not least the HIV related stigma.

Despite considerable efforts there is still no vaccine for HIV. However, the last years, after the functional cure of the Berlin patient, who was declared free of the virus after having received two stem cells transplantations against acute myeloid leukaemia (AML), from a HLA-matched, unrelated donor homozygous for the CCR5Δ32 mutation, in 2007-2008 [67], there is now the hope of a cure.

The mechanisms for HIV persistence are several, all now being addressed in therapeutic research, with the unifying theme to reduce the HIV reservoir size [68]. Most likely success will require a combination approach like early ART, agents to overcome viral latency, therapies that will strengthen the immune response to target the productively infected cells and gene therapy, or intensified ART, to protect CD4+ T-cells from infection [69].

Early diagnosis is thus also of importance for the possibility of a cure…

2.9 THE CONTINUUM OF CARE

In 2005, nearly a decade after the introduction of the highly efficient cART, attention was drawn to the fact that this achievement alone was not good enough and the population effectiveness of ART far from sufficient [70]. A model with all the needed steps to reach a well treated HIV population was described [70] and then reinforced by Ulett at al describing a "Blue-print for HIV treatment success" [71], which highlighted the equal importance of identifying the patients (diagnosis), successful linkage to care, access and acceptance of ART, adherence and retention in care.

This model, later to been known as "the HIV care continuum" or the "treatment cascade" received a lot of attention in 2011 when it was reported that in such a resource rich country as the US only 19% of PLWH reached the goal of an undetectable viral load [72] (*Figure 4*).

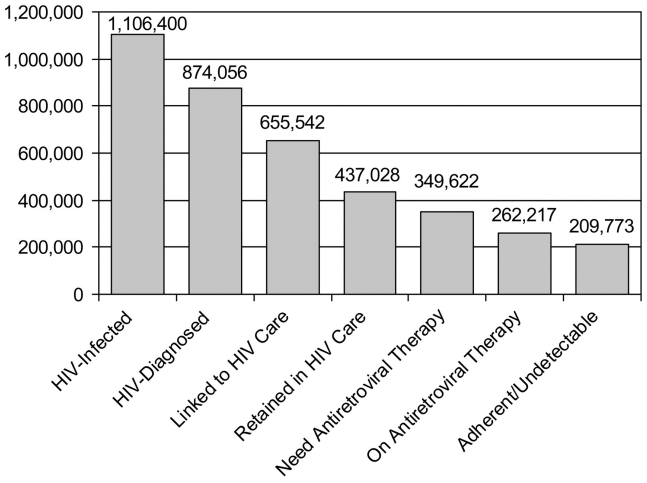

Figure 4. *The spectrum of engagement in HIV care in the United States, from HIV acquisition to full engagement in care, receipt of antiretroviral therapy, and achievement of complete viral suppression. By Gardner et al, 2011* [72].

In analogy with this the UNAIDS formulated a global "90-90-90" target in 2014, aiming at 90% of PLWH diagnosed, 90% of those diagnosed receiving ART and 90 % of those receiving ART being virally suppressed by 2020 [73].

2.10 HIV IN THE GLOBAL PERSPECTIVE

At present there are approximately 37 million people living with HIV (PLWH) worldwide (*Table 1*) and more than 34 million individuals are estimated to have died from AIDS related causes since the diagnosis of the first case of HIV in 1981. Still accounting for 1.2 million deaths in 2014 HIV/AIDS rank among the top ten of death causes globally today [74-76].

The majority of PLWH, 70%, live in the sub-Saharan Africa (SSA) and 14 % in the Asia and Pacific region, compared to in total 7% in Western and Central Europe and North America. Whereas many of the countries in SSA have generalized epidemics and heterosexual transmission dominates, sex between men are proportionally more common as transmission route in most western countries. In Eastern Europe & Central Asia the epidemic is primarily driven by heterosexual transmission and PWID whereas PWID and sex workers dominate among the newly diagnosed in Asia and the Carribean. In the Middle East and North Africa PWID, MSM and to a lesser extent sex workers dominate and in Latin America the most affected populations are MSM, followed by sex workers [74-77].

Thanks to the development of ART together with the "2000 United Nations Millennium Declaration" [78] and the establishment of initiatives like "The Global Fund to Fight AIDS, Tuberculosis and Malaria" [79] and "The United States President's Emergency plan for AIDS relief" (PEPFAR) [80] major achievements have been made. There is now a continuous decline in AIDS related mortality and the incidence of new infections has similarly declined by 35% since 2000. Still, approximately 2 million people became newly infected in 2015 and only about 40% of adults and one third of children with HIV, are receiving ART globally [81]. Considering the new "treat all" recommendation [82] and taking into account that the delivery of ART only represents one part of the "treatment cascade" (*see 2.9*) this is far from sufficient. Also HIV testing reach is limited and there are estimates indicating that almost half of all individuals living with HIV worldwide still remains to be diagnosed [83].

The relatively greatest accomplishments this far have been made in low and middle-income countries, but whereas many of the SSA countries have found measures to control their epidemic this is not true for Central Africa and other regions in the world. The proportionally largest increase of new infections in recent years has been seen in the Middle East and North Africa, but neither in Eastern Europe, with an increase particularly among PWID and their sexual partners [84], there are signs of a declining epidemic [83]. Also in Western Europe an increase in certain populations, like MSM and older PWID, have been reported [85]. In the whole WHO European area the highest number ever, with > 140 000 newly diagnosed, was reported in 2014 [86].

Apart from high-risk groups, such as MSM and PWID, migrants from countries with a high HIV prevalence, primarily the SSA, constitute a substantial proportion of PLWH in Europe [87, 88]. Regional differences are seen and the contribution of migrant population to

national epidemics is diverse with proportions ranging from 75% migrants in Sweden to less than 5% in Poland, Slovakia, Romania, Lithuania and Estonia [89]. The majority of these migrants are presumed to have acquired their infection in their home country. However, data on HIV among the migrant population is scarce. Particularly there is limited data on HIV incidence and HIV acquisition after arrival in the new country [90].

With increased travels and migration, not the least due to the refugee situation the last year, it is important to find measures for a good surveillance of the epidemic, something that I focus on in Paper IV.

Region	Total n (%) PLWH	Newly Infected	Adult Prevalence	Deaths due to AIDS
Sub-Saharan Africa	25.8 million (70%)	1.4 million	4.8%	790 000
Middle East and North Africa	240 000 (<1%)	22 000	0.1%	12 000
Asia and the Pacific	5.0 million (14%)	340 000	0.2%	240 000
Latin America	1.7 million (5%)	87 000	0.4%	41 000
Caribbean	280 000 (<1%)	13 000	1.1%	8 800
Eastern Europe and Central Asia	1.5 million (4%)	140 000	0.9%	62 000
Western and Central Europe and North America	2.4 million (7%)	85 000	0.3%	26 000
Global Total	**36.9 million (100%)**	**2.0 million**	**0.8%**	**1.2 million**

Table 1. *Global HIV statistics 2014, adapted from UNAIDS [91].*

2.11 HIV IN SWEDEN

2.11.1 The epidemic

In Sweden the first patient with AIDS was diagnosed in 1982 [92]. In retrospective analysis however, samples stored from a hepatitis A outbreak in MSM in Stockholm in 1979-80, have been found positive for HIV indicating the start of the Swedish epidemic [93]. Additionally, also in retrospective analysis, one hospitalized patient from a highly endemic country, returning home after release from hospital, was found to have been HIV-1 infected already in 1976 (*Sönnerborg, personal communication*).

Since diagnostics became available in 1985 there has been a mandatory case reporting of HIV and AIDS, regulated in the Swedish Communicable Disease Act [94]. These reports and the personal identity number, assigned to all Swedish citizens, have allowed for a good surveillance of the epidemic. However, the usage of a non-unique code, have involved some difficulties, particularly with the risk of duplicates. Today >99% of patients in Sweden are additionally registered in the national HIV Cohort, the InfCare HIV, which is equally a clinical support decision tool, a national quality register and a research database, assuring a very high quality standard of data (for *further info see 5.1.2.2*).

After an initial peak of cases in the mid 80s, as a natural consequence of the possibility to test for HIV, the notified incidence dropped markedly and remained around 250 cases yearly until the 2000s, where after there has been an increase and around 400-450 cases yearly. The number of AIDS cases increased constantly until 1996, but has then decreased, as a result of ART. Whereas most patients affected in the beginning of the epidemic were MSM, soon followed by PWID, the majority of new diagnoses since the 90s have been made in migrants, primarily from high prevalence countries, reported to have been infected already before arrival.

At present there are 6963 patients followed at Swedish clinics corresponding to a prevalence of 0.7/1000 inhabitants (*Inf Care 2015-12-31*). Altogether 11 247 patients have been reported to be HIV infected, out of which approximately 2600 have a reported AIDS-diagnosis and approximately 2400 are deceased (*Public Health Agency of Sweden 2014-12-31*).

2.11.2 Demography

The majority of PLWH in Sweden today are male, just above 60%. About half of the patients are infected heterosexually and one third bi/homo-sexually. 6% are infected through intravenous drug use. Just above half of the patients live in one of Sweden's three major cities; the vast majority in Stockholm. Around 40% are born in Sweden, followed by one third from SSA (mainly Eritrea, Ethiopia, Uganda, Somalia, Burundi and Kenya), 10% from Asia/Pacific (mainly Thailand), 7% from Western Europe/North America, 4% respectively from East Europe/Central Asia and The Caribbean/Latin America and 2% from North Africa/Middle East (*data obtained from InfCare HIV database*). As a comparison around 15% of the total Swedish population are born abroad [95].

While the reported domestic infections have remained fairly constant over the years the variation in incidence has mainly been attributable to variations in migration patterns. The last years the number of reported domestic infections has slightly decreased. (*Figure 5a-b*).

An important explanation for this is the high proportion of patients on ART, but most likely, to some extent, also the development of needle exchange programs for PWID. However a risk of underestimating the number of migrants infected after arrival has been reported and needs to be further assessed [96].

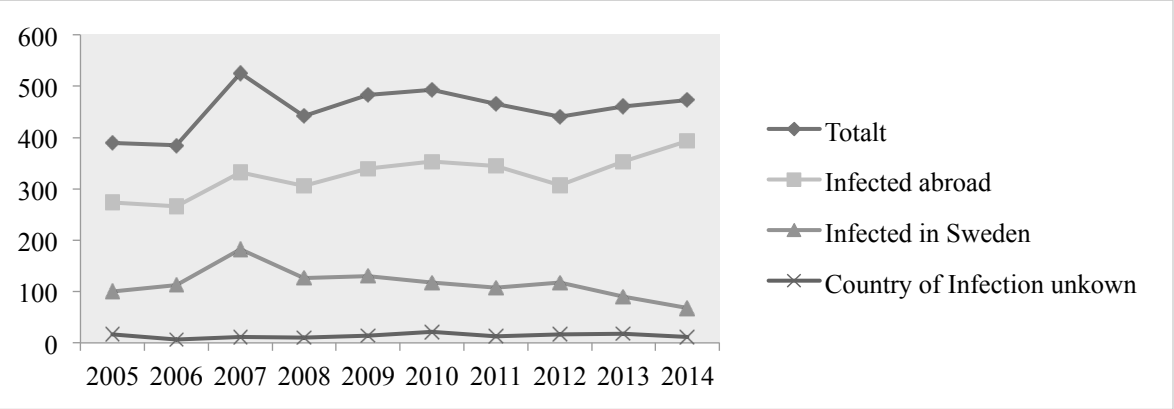

Figure 5a. *Newly reported HIV cases in Sweden, 2005–2014, divided by infected in Sweden and abroad. (Data provided by Maria Axelsson, Public Health Agency of Sweden).*

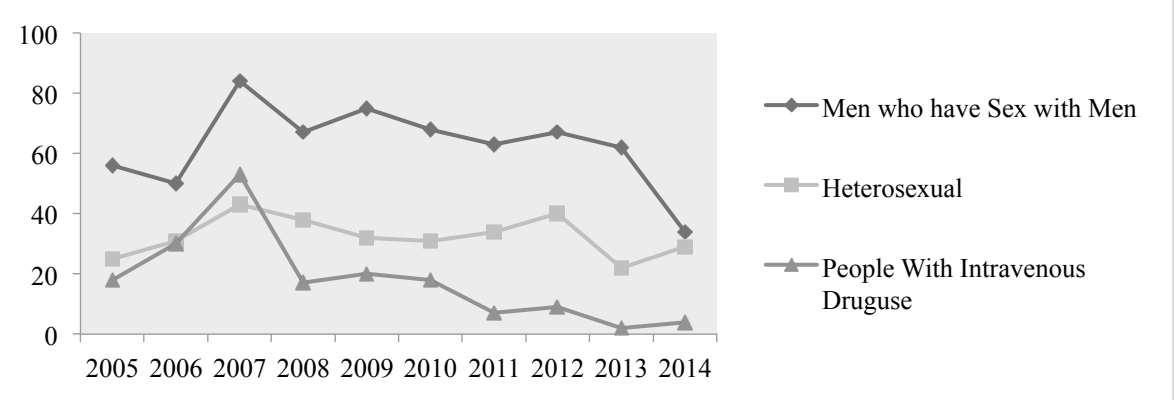

Figure 5b. *Newly reported HIV cases in Sweden infected in Sweden, 2005–2014, divided by route of transmission. (Data provided by Maria Axelsson, Public Health Agency of Sweden).*

2.11.3 Testing, treatment and care

Free and anonymous HIV-testing and care have been available throughout the epidemic and since 2004/2005 also free ART was made available by the law of communicable diseases.

2.11.3.1 Testing

Diagnosis is made primarily at STI clinics, Infectious Disease Clinics and by general practitioners, which contribute with around 30, 20 and 10% respectively. Around 6% are diagnosed through migrants' health and 4% through maternal clinics. Approximately 10% are diagnosed abroad (*Public Health Agency of Sweden, personal communication*).

For migrants, from countries with a high prevalence of HIV, an offer of HIV-testing was stated as a prioritized task by The Swedish National Board of Health and Welfare already in

1988. From 2008 the county councils are obliged by law to offer health examinations, including an opt-out offer to test for HIV, to all asylum seekers (*Lag om hälso- och sjukvård åt asylsökande m fl. SFS nr: 2008:344 (2008-05-22*). Additionally, there is the "National strategy against HIV and AIDS" (*Proposition 2005/06:60*), including the aim to identify possible HIV infection among all asylum seekers and newly arrived family connections within two months after arrival to Sweden. To what extent this has been obeyed was at the initiation of these studies unknown.

2.11.3.2 Treatment and the continuum of care

Diagnosed patients are treated and followed at Infectious Disease Clinics and one STI clinic. Altogether there are 30 specialized clinics distributed all over the country. More than half of the patients (55%) are treated at in total four clinics, in the three largest cities, Stockholm, Gothenburg and Malmö. 17% of the patients are treated at the Karolinska University Hospital. The care and treatment are multi-professional and involves physicians, nurses and as well as counsellors. If needed there are also psychiatrists, specialist in addiction medicine and maternal health with close collaboration with the team.

Treatment is considered to all patients, disregarding the CD4+ T-cell count, and initiated as soon as the patient is prepared for this. The patients are normally monitored 2-3 times per year, where the HIV RNA Viral Load (VL) is the single most important marker for treatment success. Additionally, the CD4+ T-cell count and regular blood chemistry are followed.

There is access to all treatment regimens, approved by the Swedish Medical Products Agency, and well established national recommendations on what treatment regimens to use, also including health economical aspects [97]. Care is primarily patient-centred and treatment always individualized.

Thanks to the well-developed surveillance system, described above, the linkage from testing sites to HIV Care is no major problem and a recent analysis, based on the InfCare HIV, showed that as many as 99.8% of all individuals diagnosed with HIV were linked to care [98]. The vast majority, 95%, were receiving ART out of which 94.5% had an undetectable viral load. Retention in care was high with 97.3% of the patients having been in contact with their respective clinic the last year. Overall mortality is 1% per year, which is comparable with the mortality in the Swedish population as a whole [95].

Even though further improvements are always possible there is thus not much to be improved regarding linkage, ART, adherence and retention in care and Sweden has recently been declared to be the first country in the world to reach the UNAIDS "90-90-90" [99], with 90% diagnosed, out of which 90% on sustained ART and 90% of these with viral suppression [73]. However, the figure of 10% undiagnosed included in this report is a very rough estimate and there are on going studies indicating that the true number is somewhat higher (*Sönnerborg, personal communication)* and other assessments reporting figures of 10-20% (*see 3.2 The hidden epidemic*). Regardless of what the true number is, we do know that a majority of our patients are diagnosed at a late stage of infection and that people still develop and die from AIDS in Sweden today. To me, this implies that we definitively could improve the first step of the cascade, which is also the main focus of this thesis.

3 LATE HIV DIAGNOSIS – LATE PRESENTATION

3.1 LATE DIAGNOSIS OF HIV-1 INFECTION

With its detrimental effects on the health of the individual and its societal burden, in terms of increased risk of transmission and high costs, late diagnosis of HIV-1 infection has attracted more and more attention as a central problem in the HIV-1 epidemic [100-106]. In the absence of a vaccine and cure, the undiagnosed individuals have been described as the biggest challenge yet in the fight against HIV/AIDS in the Western world [48], where, as a comparison, a timely initiation of treatment today enable a healthy, reproductive life and a near to normal life span [50, 107-109].

My interest in the late diagnosis of HIV infection started already in 2003, when I took the initiative to Paper I. At that time there were limited reports in the field and only a few studies covering the years after introduction of cART [110-113]. To manage the disease, the treatments and its side effects had absorbed a lot of attention and time, but with modern drugs and all requirements for a successful treatment fulfilled the incitements to get tested had now increased and patients still developing AIDS was seen as a failure.

In 2006, ten years after the introduction of cART, the CDC started to recommend routine, opt-out, HIV screening in all individuals aged 13–64 presenting for care in any health care setting in the United States in order to enhance early diagnosis [114]. This meant offering a HIV-test to every patient, but with the option to deny, rather than pin pointing specific risk groups. The effectiveness of this kind of general testing has however been debated [115, 116].

In Europe key stakeholders from civil society, health care and European public health institutions gathered in Brussels in 2007 and formed a pan-European platform with the objective to improve early diagnosis and earlier care of HIV across Europe; the HIV in Europe initiative [117]. Ahead of this meeting information had been gathered by reviewing existing literature and found evidence of between 15 and 38% of all HIV cases being diagnosed late in Europe [100]. A large number already severely immune-compromised at diagnosis naturally also implied a hidden epidemic with a great number of people still not yet diagnosed.

3.1.1 A pilot study at the Karolinska University Hospital

In parallel with the HIV in Europe initiative, in which we also participated, we formed a group at Karolinska University Hospital aiming at improving early diagnosis in Sweden. In an early pilot study at our clinic in 2007 [118] it was shown that 34 of 82 (41%) of newly diagnosed patients had an advanced HIV disease (CD4+ T-cells < 200/mm^3) and that 38% of these were diagnosed with the indicator diseases hepatitis B and C, TB or STI, without a HIV-test, prior to HIV diagnosis, indicating a doctor's delay. The 1-year mortality among the 34 was as high as 12%. 71% were migrants from outside of the Nordic countries, out of whom 96% were estimated to be infected before arrival to Sweden, as determined by the doctors interview with the patient. These findings indicated an obvious need of further studies.

3.1.2 The Late Presenter

The definition of what is meant by "late" in the context of HIV-1 diagnosis has been diverse. Whereas some studies have used laboratory-based definitions, such as CD4+ T-cell counts below certain thresholds, others have used clinical definitions based on time to the development of AIDS. All of them have followed the same theme; that the diagnosis is late if the patients already should have commenced ART [100], but there was a need of a joint definition to enable comparisons.

Based on results from the UK CHIC study and further endorsed by the two initiatives "the HIV in Europe initiative" and "late Presentation for HIV treatment in Europe program" a consensus definition was agreed upon and published in 2011 [106]. A so-called **late presenter (LP) was defined as an HIV positive individual presenting for HIV care with a first CD4+ T-cell count <350 cells/mm^3 or AIDS** [2]. A subgroup of the individuals most severely immune-compromised, with **CD4+ T-cell count <200 cells/mm^3 or AIDS, was defined as advanced HIV** [2]. (*Figure 6*).

This was all in line with the treatment guidelines at the time, which stated that all patients with a CD4+ T-cell count <350/mm^3 should receive ART even if asymptomatic [119].

Late presentation for care, per definition, includes both the problem of late diagnosis and poor linkage to care after HIV diagnosis. As a contrary to what has been described in other studies [71, 72] linkage is not a major issue in Sweden (*see 2.11 HIV in Sweden*) and the focus of my thesis are subsequently patients with a late HIV-1 diagnosis.

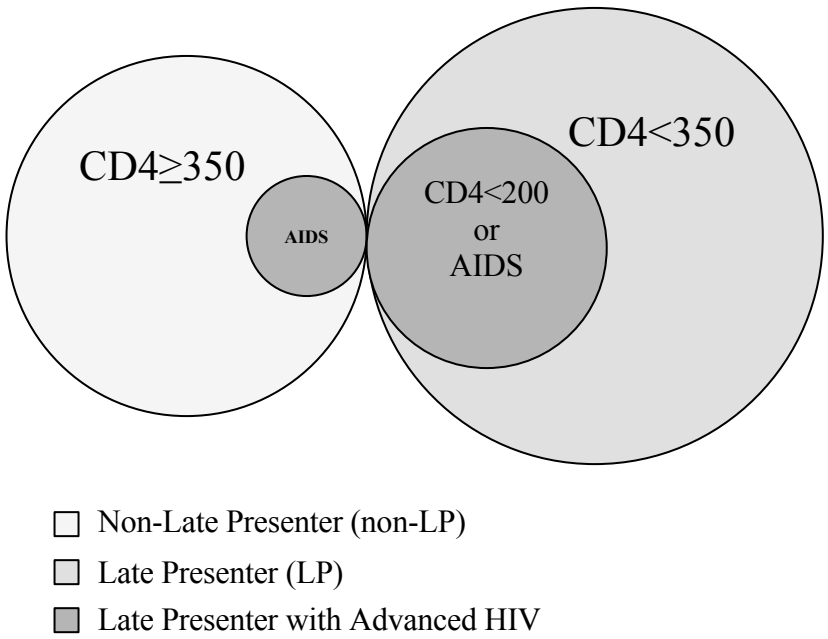

Figure 6. *The definition of a Late Presenter according to the European Consensus definition.*

3.2 THE HIDDEN EPIDEMIC – ESTIMATING THE PROPORTION UNDIAGNOSED

Globally, as mentioned, estimates from the UNAIDS, suggest that almost half of all PLWH still remain to be diagnosed [83]. In the WHO Europe region there are estimates of 30-50% undiagnosed [120], with proportions ranging from 12-20% in Scandinavia to just above 50-60% in some Eastern European countries and Russia [99, 120, 121]. Numbers reported from the US are similar to those in Western Europe, with up to one-quarter of the HIV positives undiagnosed [122]. Rough estimations from Sweden have reported numbers of 10-20%. These have been based on the prevalence of patients with simultaneous HIV and AIDS-diagnosis (10-12%), a low prevalence of non-diagnosed HIV in maternal and blood donor screening (1/10 000) and a large proportion of migrants among the late diagnosed, expected to have spent most of their time unaware of their diagnosis abroad [123].

To get accurate estimates of the size of the HIV epidemic is important to facilitate plans for HIV-testing and the corresponding treatment and also to evaluate national prevention and treatment programs. There are several ways to do this, historically most commonly by using prevalence surveys [124]. There are also methods based on estimations of cumulative incidence of HIV infections [125-129] and those based on surveillance of simultaneous HIV/AIDS cases, like the London method [130].

To be able to make as good estimations as possible it is recommended to collect data on HIV diagnosis, CD4+ T-cell count at diagnosis, proportion with simultaneous HIV/AIDS and data on deaths in people with HIV [124].

Many of the models have required quite advanced modelling, but recently the ECDC published a tool, based on the Incidence method and the London method, of simultaneous HIV/AIDS cases, that is possible for anyone to download as a desktop application (*http://ecdc.europa.eu/en/healthtopics/aids/Pages/hiv-modelling-tool.aspx*). By introduction of basic surveillance data to these, estimates of both the proportion not yet diagnosed and the average time between infection and diagnosis can be received.

While above-mentioned methods all are based on surveillance data there are also models based on serological assays, such as the BED-test (HIV-1 IgG capture BED enzyme-linked immunoassay) with which the HIV incidence can be calculated by detecting seroconversions as a sign of recent infection (approximately within six months) [131, 132]. Additional algorithms, such as the RITA (recent infection testing algorithm) [132, 133], use the BED-test together with the CD4+ T-cell count and clinical information, like on going ART, and/or an AIDS-defining illness taken into account [132].

All methods produce valuable, but rough estimates and refined tools would make a valuable contribution in estimating the true proportion of persons living with an undiagnosed HIV infection. Important prerequisites are naturally the country involvement and national surveillance systems that are operating satisfactory.

3.3 CONSEQUENCES OF THE LATE DIAGNOSIS

3.3.1 Increase in mortality and morbidity

3.3.1.1 Mortality

Late presentation of HIV-1 infection is associated with severe and sometimes fatal consequences for the individual and in regions with full access to ART, a delay in diagnosis is now being a dominating reason for HIV related death [3, 134-136]. In total late diagnosis has been described to account for one third of all HIV related deaths and as much as one quarter of all deaths in HIV positive individuals in a western country [100]. Most of the deaths in HIV-1 patients occur within the first year after diagnosis and over 90%, of this short-term mortality, can be related to a late diagnosis per se. Comparing the mortality in the newly diagnosed LP with those diagnosed early there is at least a 10-fold increased risk of dying within the first year (4.1% versus 0.3%) [137] and the more immunosuppressed naturally the higher risk [136].

Also long term mortality is affected and patients with a CD4+ T-cell count < 200 cells/mm^3, when they commence ART, have been estimated to have a life expectancy, at 20 years of age, that is at least ten years less than that in those who initiate ART when the CD4+ T-cell count had just fallen below 350 [107]. Late diagnosis and treatment start at lower CD4+ T-cell counts are associated with a poorer immune recovery [138, 139], but if there is a significant raise in the CD4+ T-cell count, after starting ART, and levels >500/mm^3 are reached, the mortality is similar to that of the general population for non-PWID. However, regardless of a gain in CD4+ T-cells, even above 500/mm^3, an increase in mortality remains for those with a previous AIDS defining illness [46].

3.3.1.2 Morbidity

Late presentation and treatment start at low CD4+ T-cells count are associated with an increased risk of both HIV- and non-HIV-related morbidity such as opportunistic diseases, AIDS- and non-AIDS-defining cancers as well as cardiovascular, hepatic and renal disease [3] and as such also premature aging [140]. Most likely this is due to larger latent viral reservoirs [141] and a higher degree of chronic inflammation [65, 66] in these patients.

Also, patients presenting late have been proven to be more difficult to treat, since there, in addition to the blunted or delayed CD4 response, is an increased risk of drug-drug interactions, toxicity and the immune reconstitution syndrome [142].

Even though different cut-offs of CD4+ T-cells have been discussed studies, like the SMART study, have indicated that there is no clear threshold, but rather a continuum of increased risk for both disease progression and death associated with lower CD4+ T-cell counts [143, 144]. In the spring 2015 the START trial also showed a clear advantage of immediate ART, disregarding the CD4+ T-cell count, with a significant reduction of AIDS and death in those starting with CD4+ T-cell counts >500/mm^3 compared to those waiting until 350/mm^3 [58], making the early diagnosis increasingly important.

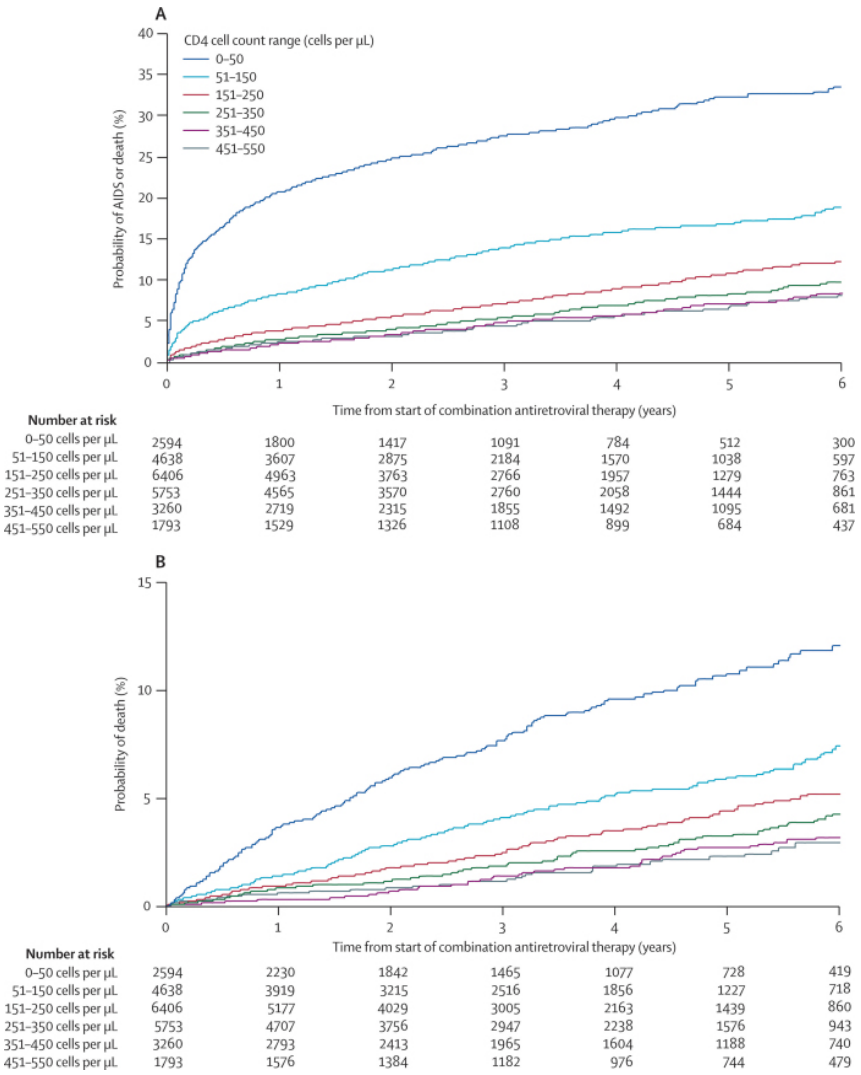

Figure 7. *Cumulative probability of (A) AIDS or death or (B) death alone after initiation of combination antiretroviral therapy, according to range of CD4 cell count at the time of treatment initiation. JA Sterne et al, Lancet 2009 [145].*

3.3.2 The risk of onward transmission

Since the average time from HIV infection to symptomatic disease is as long as 8-10 years, without treatment [44], there are several years when an undiagnosed carrier accidently, and completely unaware, could be transmitting the virus to others, most prominent at PHI and at the late stages of infection [146, 147]. Estimations show that in an HIV population, where 25% are unaware of their infection, these count for 54% of all new transmissions, corresponding to a 3.5 times higher likelihood to transmit the virus [4].

Awareness of HIV-infection is per se associated with modified sexual behaviours [148-151], but most important for the reduced infectivity of HIV is the use of ART, which reduces a person's infectiousness by reducing plasma and genital HIV viral loads [152, 153]. One of the earliest and best documented proofs of this is the dramatic reduction seen in mother-to-child transmission, where in Europe the risk of vertical transmission has decreased from 15.5% in 1994 [104] to virtually zero in those well treated today [154].

As for sexual transmission scientific data unambiguously show that ART substantially reduces the contagiousness of HIV, as well at a population [155] as at an individual level, where the landmark study, HPTN 052, showed a 96% risk reduction of transmissions in heterosexual serodiscordant couples, when the HIV-positive partner received ART [156]. Also the ongoing Partner study, reports no transmissions after nearly 890 couple years of follow up in 586 heterosexual and 308 MSM serodiscordant well treated couples [157], presenting the first evidence of a risk reduction also for MSM. Even though it is scientifically still not possible to state a null-risk and longer follow up is needed; as it is stated in the "Swiss statement" [152], many experts now consider a HIV positive individual, with undetectable viral load for at least 6 months and without other sexual transmitted diseases (STIs) to have minimal contagiousness [35].

During recent years there have been a lot of discussions about treatment as prevention (TasP) and the "test and treat" strategy aiming at eliminating the HIV epidemic by annual universal testing followed by immediate ART if diagnosed positive [158]. Even though this does not seem realistic in the short term, early diagnosis and ART, in the absence of a protective vaccine, is the most promising strategy to control the pandemic.

3.3.3 Health economical aspects

At a societal level late presentation is associated with a direct and indirect burden on health economy [159].

Whereas the total cost is hard to assess, there are reports of the direct medical cost. One large US study demonstrated the later to be significantly higher in late presenters compared to those initiating treatment with a CD4 > 350 and remaining twice as high the subsequent years despite significantly improved CD4 [159]. Additionally, another US study also demonstrated higher direct medical costs continuing even after 7 to 8 years in care [160].

One should also keep in mind the cost before the diagnosis of the patient where many patients are the subjects of comprehensive, often unnecessary and sometimes also invasive investigations before someone even think of HIV [161].

3.4 WHO ARE THE LATE PRESENTERS?

The characteristics of Late Presenters differ in different regions of the world, in different countries and even in different cities and settings and late presentation has been observed to be common in all demographic groups [162]. However some main common features have been identified; Late presenters tend to be those who do not perceive themselves, or are not perceived to be, at risk of HIV infection, those who are not routinely offered HIV testing, and are often from marginalized groups [104].

At the initiation of my study some studies had also identified an association with older age [110, 163] and foreign birth [110, 163, 164] as risk indicators for late diagnosis, whereas results regarding gender and transmission groups had been conflicting [162].

The situation in Sweden was at the time not known and studies assessing this needed.

3.5 MISSED OPPORTUNITIES FOR TESTING

With a latency phase of several asymptomatic years before getting ill [44] it is understandable that many HIV infected patients do not seek health care until late stages of their infection. However several individuals do seek health care facilities for HIV associated conditions, so called indicator diseases [165], both during a symptomatic primary infection and later on, without getting tested for HIV [166-169]. People also attend health care services for different reasons, when an epidemiological risk factor could indicate that a HIV-test would be adequate, disregarding the reason for the medical contact.

In addition to this there are the political challenges related to immigration, where the practice to perform health examinations, including HIV test, often are inadequate. Finally, there is also the delay of the patient in seeking health care.

All the above represent missed opportunities for testing and consequently also missed opportunities for treatment and prevention.

One of the main aims with this thesis was to assess the extent of missed opportunities in a Swedish context.

3.6 INDICATOR GUIDED TESTING

As a contrary to the general opt-out HIV testing, proposed by the CDC in the US [114], most countries in Europe, through the initiative HIV in Europe [117], have favoured a strategy of targeted testing. Since the historically predominant strategy, based on risk factors, have failed to identify a substantial proportion of PLWH, focus is now aimed at identifying any patient presenting with conditions that could be indicative of HIV, irrespective of previous risk assessments [165]. These conditions include:

- All conditions that would be AIDS-defining in PLWH
- Other HIV associated conditions
- Conditions not associated to HIV, but where HIV is important to rule out to prevent future complications in the clinical management of the patient.

The first and the last (including mainly conditions needed to be treated with different kind of immunosuppressive therapy) are rather straightforward whereas the HIV associated conditions could be many and are less well defined. These include infections that share a common mode of transmission with HIV (e.g. hepatitis, sexually transmitted infections (STI)), diseases who are favoured by the immunodeficiency induced by HIV (e.g. oral thrush, zoster (VZV)) and any other medical condition associated with an untreated HIV infection (e.g. fever of unknown origin, blood dyscrasia). For a more detailed list on indicator conditions, see *Table 2* on page 26-27.

In health economic studies from France and the US cost effectiveness of general screening have been demonstrated at an undiagnosed HIV prevalence of 0.1 % [170, 171], in one study

as low as 0.05% [172]. The prior has been used as a rule of thumb of when it is indicated to screen for HIV and utilized for the HIV associated conditions, but also to decide on when screening is indicated within targeted groups and in the general population. For example in the UK HIV testing is recommended by GPs in all newly registered individuals 15-59 years in areas with a diagnosed HIV prevalence >/=2 /1000 inhabitants [173].

3.7 BARRIERS TO HIV TESTING

Barriers to testing are several, but my personal clinical experience is that a patient very seldom says no when getting an offer to test. This has also been confirmed in studies, where the patient acceptability has been >90% and a low testing rate rather have been due to staff generated barriers [166, 174]. Barriers among health care professionals have been described to include lack of education and self confidence [175], discomfort [176], concerns about the consent process and competing priorities [177, 178], but also failure in risk assessments [179]. For the patients well-known barriers include low risk perception of HIV infection, structural barriers, fears of the disease or of negative consequences, such as HIV-related stigma and concerns about confidentiality [175, 180].

Patient's barriers to HIV testing are being assessed by our research team but are not within the scope of this thesis.

Table 2. Indicator conditions for HIV infection and specialties involved.

Adapted from Lazarus et al. HIV Med 2013 [165].

1. ***Dentistry***

- *Oral hairy leukoplakia*[2]
- *Candidiasis, oral and oesophageal*[1]*
- *Kaposi's sarcoma*[1]*

2. Dermatology/dermatovenereology/genitourinary medicine

- *Kaposi's sarcoma*[1]*
- *Herpes simplex ulcer(s)*[1]*
- *Atypical disseminated leishmaniasis*[1]*
- *Penicilliosis, disseminated*[1]*
- *Seborrheic dermatitis/exanthema*[2]*
- *Herpes zoster*[2]*
- *Sexually transmitted infections*[2]*
- *Hepatitis B or C (acute or chronic)* [2]*
- *Severe or recalcitrant psoriasis*[2]
- *Candidaemia*[2]
- *Candidiasis*[2]

3. Gastroenterology/hepatology

- *Cryptosporidiosis diarrhoea, >1 month*[1]*
- *Microsporidiosis, >1 month*[1]*
- *Isosporiasis, >1 month*[1]*
- *Candidiasis, oesophageal*[1]*
- *Hepatitis B or C (acute or chronic)* [2]*
- *Unexplained chronic diarrhoea*[2]**

4. Gynaecology/obstetrics

- *Cervical cancer*[1]*
- *Sexually transmitted infections*[2]*
- *Hepatitis B or C (acute or chronic)* [2]*
- *Pregnancy (implications for the unborn child)* [2]
- *Cervical dysplasia*[2]*

5. Haematology

- *Lymphoma, non-Hodgkin*[1]*
- *Malignant lymphoma*[2]
- *Unexplained leukocytopenia/thrombocytopenia lasting >4 weeks*[2]*
- *Thrombotic thrombocytopenic purpura*[3]

6. Infectious diseases/internal medicine

- *Tuberculosis*[1]*
- *Mycobacterium tuberculosis, pulmonary or extrapulmunary*[1]*
- *Mycobacterium avium complex (MAC) or Mycobacterium kansasii, disseminated or extrapulmonary*[1]*
- *Mycobacterium, other species or unidentified species, disseminated or extrapulmunary*[1]*
- *Pneumonia, recurrent (two or more episodes in 12 months)* [1]*
- *Pneumocystis jirovecii pneumonia*[1]*
- *Cryptococcosis, extrapulmonary*[1]*
- *Salmonella septicaemia*[1]*
- *Cytomegalovirus, other (except liver, spleen or glands)* [1]*
- *Herpes simplex ulcer(s) >1 month with bronchitis/pneumonitis*[1]*
- *Candidiasis bronchial/tracheal/pulmonary*[1]*
- *Candidiasis, oesophageal*[1]*
- *Histoplasmosis, disseminated/extrapulmonary*[1]*
- *Coccidiodomycosis, disseminated/extrapulmonary*[1]*
- *Atypical disseminated leishmaniasis*[1]*
- *Reactivation of American trypanosomiasis (meningoencephalitis or myocarditis)* [1]*
- *Penicilliosis, disseminated*[1]*
- *Sexually transmitted infection*[2]*

- *Hepatitis B or C (acute or chronic)[2]**
- *Mononucleosis-like illness[2]**
- *Invasive pneumococcal disease[2]*
- *Herpes zoster[2]**
- *Lymphocytic meningitis[2]*
- *Visceral leishmaniasis[2]*
- *Unexplained weight loss[2]***
- *Unexplained fever[2]**
- *Unexplained chronic diarrhoea[2]***
- *Unexplained lymphadenopathy[2]**
- *Unexplained leukocytopenia/thrombocytopenia lasting >4 weeks[2]**

7. Nephrology

- *Unexplained chronic renal impairment[2]*

8. Neurology and neurosurgery

- *Cerebral toxoplasmosis[1]**
- *Cryptococcosis, extrapulmonary[1]**
- *Progressive multifocal leucoencephalopathy[1]**
- *Reactivation of American trypanosomiasis (meningoencephalitis or myocarditis)[1]**
- *Guillain–Barré syndrome[2]*
- *Mononeuritis[2]*
- *Subcortical dementia[2]*
- *Multiple sclerosis-like disease[2]*
- *Peripheral neuropathy[2]*
- *Primary space-occupying lesion of the brain[3]*

9. Oncology

- *Lymphoma, non-Hodgkin[1]**
- *Kaposi's sarcoma[1]**
- *Primary lung cancer[2]*
- *Anal cancer/dysplasia[2]**
- *Cancer requiring aggressive immunosuppressive therapy[3]*

10. Ophthalmology

- *Cytomegalovirus retinitis[1]**

11. Otorhinolaryngology

- *Candidiasis tracheal/oesophageal[1]**
- *Mononucleosis-like illness[2]**

12. Rheumatology

- *Autoimmune disease treated with aggressive immunosuppressive therapy[3]*

13. Respiratory medicine/pulmonology

- *Tuberculosis[1]**
- *Pneumocystis jirovecii pneumonia[1]**
- *Pneumonia, recurrent[1]**
- *Mycobacterium avium complex (MAC) lung disease[1]**
- *Histoplasmosis, disseminated/extrapulmonary[1]**
- *Herpes simplex bronchitis/pneumonitis[1]**
- *Candidiasis bronchial/pulmonary[1]**
- *Community-acquired pneumonia[2]*

14. Emergency medicine

- *Symptomatology fitting any of the listed conditions*

13. General practice

- *Symptomatology fitting any of the listed conditions*

***1** AIDS-defining conditions among people living with HIV **2** Conditions associated (or likely to be associated) with an undiagnosed HIV prevalence of >0.1% **3** Conditions where not identifying the presence of HIV infection may have significant adverse implications for the individual's clinical management despite the estimated prevalence of HIV being likely to be < 0.1%. ***Assessed in paper II-III** (** only if consistent with wasting).*

4 AIMS

The *overall aim* of my thesis was to assess key aspects related to late diagnosis and missed opportunities for timely diagnosis and care in the Swedish HIV-1 epidemic.

The *specific* aims were:

Paper I

- To analyse the characteristics of patients who were unaware of their HIV-1 infection until they developed AIDS, in the period after introduction of highly active antiretroviral therapy.
- To test the hypothesis that the proportion of patients with late HIV-1 diagnosis was increasing.

Paper II

- To assess the prevalence of Late Presenters of HIV-1 infection in Sweden, according to the new European consensus definition [2].
- To identify factors in the newly diagnosed HIV-1 infected patients and within the health care system, which contribute to late diagnosis.

Paper III

- To further characterize and identify risk factors for the missed opportunities of HIV-1 diagnosis identified in Paper II.

Paper IV

- To assess whether the number of migrants infected after arrival to Sweden is underestimated, by comparing the estimates of the country of HIV-1 acquisition using a CD4+ T-cell decline trajectory model with what was clinically reported.

5 MATERIAL AND METHODS

5.1 STUDY POPULATION AND DATA COLLECTION

All studies in my thesis were conducted in Sweden, a country with nearly 9.8 million inhabitants and an HIV-prevalence around 0.7/1000 inhabitants. For further details about the study setting, see *Background; 2.11 HIV in Sweden*.

5.1.1 Study population

In **Paper I**, the national register of HIV and AIDS at the Public Health Agency of Sweden (former Swedish Institute for Infectious Disease Control) (*see 5.1.2.1*) was used to select all patients ≥15 years old registered with an AIDS diagnosis in Sweden between 1996 and 2002.

The year 1996 was chosen in order to study the HAART era. All children under the age of 15 were excluded since AIDS development in a child rather could be attributed to factors relating to the parents than to the child per se.

For **Paper II-III**, all the four largest HIV clinics in Sweden, located in the three major cities (Stockholm, Gothenburg, and Malmö) and additional county clinics, with which the research team had an established collaboration, were approached and invited to participate. In total 12 clinics, distributed all over the country and representing 75% of the national HIV care, joined the study.

For every clinic a designated principal investigator was chosen. Together with the research team three of these formed a steering committee that had the possibility to give input on the study design before this was finalized.

At the 12 clinics all patients, ≥18 years, who were newly diagnosed with HIV-1, from 1st of October 2009 to 31st of January 2012, were included in the study. Within six months the patients were asked to participate in a sub-study based on a more detailed questionnaire.

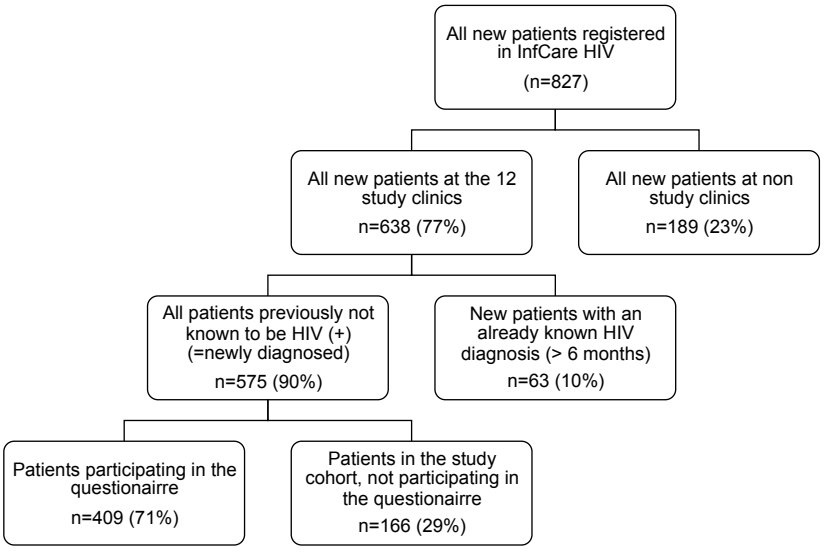

Figure 8. *Flowchart of the patients included in the Paper II-III study 1st of October 2009 to 31st of January 2012.*

In **Paper IV** all non-Swedish born individuals (all defined as migrants), aged 15 or above, with an HIV-1 diagnosis between 1983 and 2013, with a known year of arrival and without a known PHI or mother to child transmission (MTCT), registered in the InfCare HIV (*see 5.1.2.2 below)*, were included. In a sub-study, made for sensitivity analysis, we excluded all patients resident less than six months in Sweden at diagnosis.

5.1.2 Sources of data

5.1.2.1 The national register of HIV and AIDS

In Sweden there has been a mandatory case reporting of HIV since 1983 and during the period 1985-2005 also of AIDS (*National Health Protective Agency, yearly report 2014*). The cases are reported, by a non-unique patient code, to the local County Medical Officer and to the Public Health Agency of Sweden, former Department of Epidemiology at the Swedish Institute for Infectious Disease Control (EPI/SMI), where they are registered in a national register of HIV and AIDS. This register was used in **Paper I.**

At the time of the study a complementary questionnaire, collecting additional and missing information about the patient was sent to the patient's physician six months after the initial case report, and subsequently entered into the same database. This information further helped in the cleaning process of the register and minimized the possibility of remaining duplicates.

5.1.2.2 The InfCare HIV

The InfCare HIV, used in **Paper II-IV**, was initiated in 2003 and incorporates >99% of all HIV-1 infected patients living in Sweden today and an additional high proportion of deceased individuals, with diagnosis from 1983 and onwards. It is equally as a research database, a national quality register for the Swedish HIV Care and a day-to-day electronic decision support tool in the clinic (*Figure 9*), where all patients are followed from diagnosis and onwards.

It contains socio-demographic data (gender, age, country of origin, estimated country of transmission, route of transmission, date of first positive HIV serology) and biological data (CD4+ T-cell count, plasma and CSF HIV RNA, HCV and HBV serostatus), ART history and data on HIV drug resistance, including the viral sequences.

Whereas assigned medical staff registers personal data, the majority of the biomedical parameters are retrieved electronically, in real time (once a night), from the laboratory, making the database continuously up to date and of very good quality. Internal validation is assured through a quality index and external evaluations have been performed by IBM Research Haifa, the Mac Planck Institute of Bioinformatics and through the Cohere database quality assurance system (HIV Cohorts Data Exchange Protocol) (http://www.eurocoord.net).

As of 31st of December 2015 there were 10,437 patients registered in the database and 6,969 patients followed at the Swedish clinics.

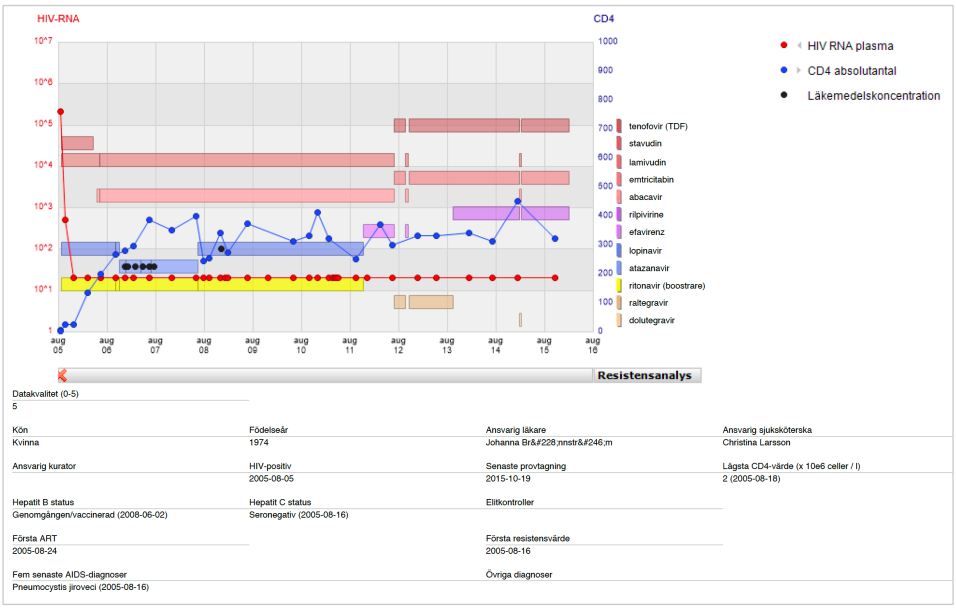

Figure 9. *Patient graph from the decision tool in InfCare HIV*

5.1.2.3 Questionnaire

For **Papers II-III** an in-house questionnaire was developed. The questions were based on an extensive literature review on late HIV diagnosis including psychosocial aspects of living with HIV as well as the involvement of experts in the area of infectious diseases, addiction medicine, public health, epidemiology and community representatives. The feasibility of the items was assessed through interviews with people living with HIV, in collaboration with the patient-organisation HIV-Sverige (former Riksförbundet för hivpositiva).

The questionnaire was completed by the treating physician and based on the medical history from the patient and on hospital journals, for those patients who agreed to participate in the sub-study. An interpreter participated if necessary. Part of the data collected was also used in **Paper IV**. For details on the data collected and used see *5.4.1 Variables* below.

5.2 THE CD4+ T-CELL DECLINE TRAJECTORY MODEL

The method for assigning probable country of HIV acquisition among migrants was first developed by Public Health England, in collaboration with the European Centre for Disease Prevention and Control (ECDC) in 2012 [96] and later revised and improved including collaboration with our research group (*In manuscript: "Probable country of HIV infection acquisition among diagnosed people born outside a country: applying a new method on four European countries")*. In summary, the method, which is based on factors associated with the intercept and the slope of CD4+ T-cell decline in a large group of seroconverters, uses a mathematical algorithm to model CD4+ T-cell decline trajectories in ART naïve patients in order to estimate their time from HIV seroconversion. Since the CD4+ T-cell count at infection varies significantly by ethnicity and the rate of CD4+ T-cell cell decline is associated both with ethnicity and age [96, 181, 182], these demographic variables were kept

in the model and adjusted for. The algorithm also considered the variability of CD4+ T-cell count at PHI [43], by excluding all CD4+ T-cell counts within the first three months.

If the estimated period of seroconversion was after the calendar year of arrival, the patient was categorized as having acquired HIV *in Sweden* and likewise *outside Sweden* if the estimate of seroconversion was prior to year of arrival. Patients for which the year of arrival was within the time estimate of seroconversion were instead categorized as *uncertain*. These estimates were then compared with the doctor's estimate, registered as country of HIV-1 transmission in InfCare HIV.

5.3 PHYLOGENETIC ANALYSIS

Whereas the CD4+ T-cell model, described above, is a public health tool that was developed to make estimates on the population level, phylogenetic analysis could be used on the individual level to determine the source of incident cases and possible transmission clusters [183].

In the Swedish HIV treatment guidelines sequencing of the HIV-1 pol gene is recommended routinely to identify any transmitted drug resistance. The sequences are transferred electronically and registered in the InfCare HIV database [28]. Using these sequences, a phylogenetic analysis was performed anonymously without having details on patients' demographics, partner history or migration history. An anonymised maximum likelihood phylogenetic tree with the general time reversible (GTR) with inverse gamma (GTR+G+I) model was conducted on main five HIV-1 subtypes (A1, B, C, 01_AE, 02_AG) for which >50 sequences were available (n=863). Sequences with complete protease (aa 1-99) and partial reverse transcriptase (aa 1-300) were only used after excluding known drug resistant mutation positions. "*Discordant*" samples of these five subtypes (n=71) were labelled as infected in "*Sweden*" or "*outside Sweden*" based on it's clustering with "concordant" (both model and doctor) sequences with >70% bootstrap support (BS). A singlet sequence or sequence clustering with other sequences with <70% BS, the sequences were termed as "*Uncertain*".

5.4 VARIABLES AND OUTCOME DEFINITIONS

5.4.1 Variables

In **Paper I**, variables collected from the national register of HIV and AIDS were sex, age, reported country of origin, country and route of transmission and date of HIV-and AIDS-diagnosis, respectively. The AIDS-diagnosis used in Sweden is the one based on the 1993 Expanded European AIDS case definition [184] and holds for all papers, I-IV.

In Papers II-IV, gender, age, reported country of origin, country and route of transmission, CD4+ T-cell counts, date of first positive HIV test and any AIDS diagnosis were collected from InfCare HIV. For migrants also the year of arrival (immigration date) were collected, when available.

In **Papers II-III** information on missed AIDS- [45] or HIV-associated symptoms or such symptoms neglected by the patient (appearing within the preceding three years), psychiatric illness, substance use, immigration date, health examination including HIV-test at immigration, the initiator of the HIV-test and the reason for testing was collected from the questionnaires. (For details on the missed HIV-associated conditions please see appendix, Paper II-III). The questionnaire data regarding origin and immigration date was also used for **Paper IV**.

For **Papers II-III**, the countries of origin and transmission were grouped into regions based on the Joint United Nations Program on HIV and AIDS **(**UNAIDS) [185] definitions for descriptive analysis, and further grouped into: *Sweden; the East* (East Europe/Asia/Pacific); *sub-Saharan Africa (SSA); Other* (Western Europe, North and Latin America, Caribbean, North Africa, Israel, Middle East) and *Unknown* for inferential analysis. For **Paper IV** the countries of origin were grouped into world region of birth: *Africa; Europe and Other* whereas the country of transmission (doctor´s estimate) was dichotomized as *Sweden* or *outside Sweden*. The country of transmission estimated by the CD4+ T-cell algorithm was categorized as *Sweden*, *outside Sweden* and *uncertain*, as specified above.

For patients born outside Sweden a ***missed opportunity to test at immigration*** was defined as immigration date ≥1st of January 1988 and HIV diagnosis >two months after immigration, if not tested negative at immigration. The reason for choosing 1988 was the recommendations from the Swedish National Board of Health and Welfare including offering of testing as a prioritized task to refugees from countries with a high prevalence of HIV that year.

5.4.1 Outcomes

In **Paper I** the outcome was ***late tester***, defined as a patient with AIDS, for whom the time interval between first positive HIV test and AIDS diagnosis was < 3 months. This time interval was chosen since it had been used in previous studies [111], but mainly because it was regarded long enough to cover any possible delay in AIDS-diagnosis after the diagnosis of HIV. All patients with 3 or more months between HIV and AIDS diagnosis were defined as ***non-late testers***.

In **Paper II** the main outcome was ***late presentation (LP)*** defined on the basis of the European consensus definition [2]; CD4 <350 cells/mm^3 or AIDS (within three months of diagnosis). As an amendment to the definition we choose to include also PHI and defined all patients with CD4 <350 cells/mm^3, who had laboratory results and/or medical history confirming PHI the previous year as a non-late presenter (nLP). The LP were further subdivided into: LP without advanced HIV disease (LPnAH): CD4 200–349 cells/mm^3 and no AIDS and ***LP with advanced HIV disease (LPAH)***: CD4 <200 cells/mm^3 or AIDS. (*Figure 10*). The CD4 count closest to diagnosis was the one used. Since we allowed for the patients to be included within 6 months, this was also the time span defined for the CD4, presumed the patient had not yet started ART.

In **Paper III** primary outcomes were two different kinds of missed opportunities of HIV testing, already described in paper II; ***Missed at presentation***, defined as a failure to diagnose a clinical indicator for HIV testing within the national medical service the three years previous to the diagnosis and ***Symptoms neglected by the patient***, defined as the presence of

any HIV or AIDS associated symptom, experienced by the patient, without seeking medical care, at all or not until additional symptoms evolved, the three years previous to the diagnosis. Secondary outcome was ***the initiator of the test***.

In **Paper IV** a variable, having as categories all the possible combinations of disagreement between the doctor and algorithm estimates, called *discrepancy* was created and further dichotomized in two groups, according to if the doctors and the algorithm agreed or not.

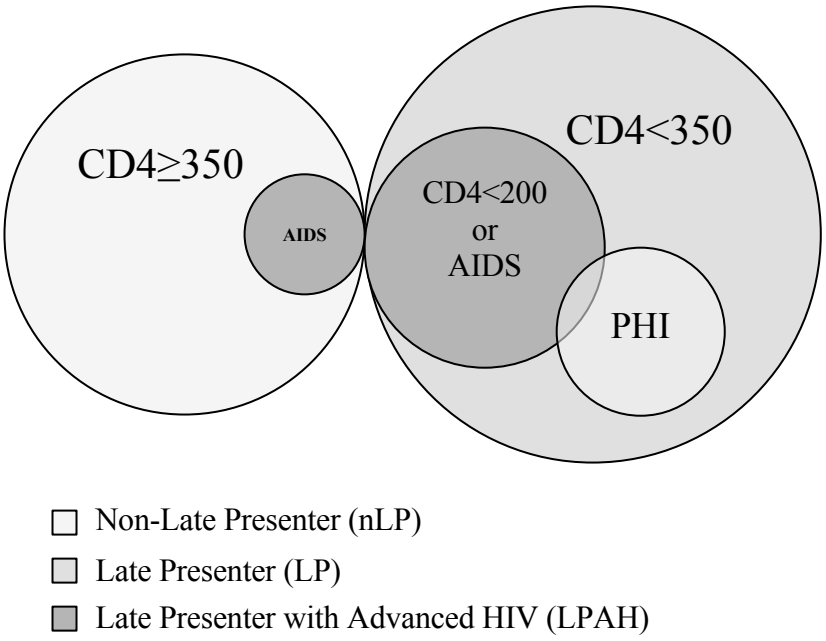

Figure 10. *The adjusted definition of the Late Presenter used in paper II-III.*

5.5 STATISTICAL ANALYSIS

In all papers data was summarized with **descriptive statistics** (mean, median, standard deviation for numerical variables, frequencies and percentages for categorical variables).

In the **bivariate analysis** cross tabulations with Chi-Square or, when numbers were small, Fisher test were used to test for un-adjusted relationship between categorical outcomes and categorical independent variables. For numerical outcomes, t-test or Wilcoxon rank sum test, and ANOVA or Kruskal Wallis test were used to compare mean and medians in two and more groups respectively.

For the **multivariable analysis,** adjusting for confounders, a binary (for binary outcomes) and a multinomial (for categorical outcomes with more then two categories) logistic regression model were used to identify predictors in **Paper II**. In **Paper III** we used the binary logistic regression whereas again, the multinomial logistic regression model was used in **Paper IV**.

Crude and adjusted odds ratios (OR) with 95% confidence intervals were presented. P-values <0.05 were considered significant in the final models.

Additionally, in order to study nonlinear associations among migrants infected abroad, in relation to number of months in Sweden, with probability of being late and type of test initiator, generalized logistic models were used, fitting restricted cubic splines for months in Sweden, choosing four knots according to Harrell's recommended percentiles, in **Paper III**.

Data analysis was done using the STATA software 13 (Stata Corp. College Station, USA).

5.6 ETHICAL CONSIDERATIONS

All four studies were conducted in accordance to the declaration of Helsinki [186] and approved by the Regional ethics committee in Stockholm (Diary number 03-170, 2009/1029-31/1-4 and 2014/928-31/2) and by the Regional ethics committee in Gothenburg (Diary number 532-11 including amendment 20111118).

6 RESULTS AND DISCUSSION

6.1 PAPER I

6.1.1 Late testers in Sweden 1996-2002

A total of 487 patients, ≥ 15 years, reported with AIDS in Sweden 1996-2002 were identified, of whom 219 (45%) were late testers and thus previously unaware of their HIV-1 infection. The incidence of late testers was fairly constant over the study-period, but their proportion of patients with AIDS increased from 22% in 1996 to nearly 60% at the end of the study. Whereas the high proportion of patients with AIDS despite an early diagnosis (non-late testers) in 1996 was a natural consequence of cART just becoming available [187], there was still a small trend towards a higher proportion of Late Testers during the following years (*Figure 11*). Similar observations had been made in other European countries [110, 111] and Australia [112], with proportion of late testers ranging from 35% to the 60% seen at the end of our study.

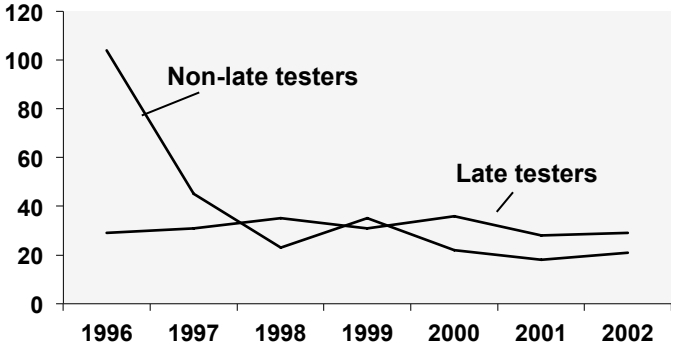

Figure 11. *AIDS cases in Sweden 1996-2002 divided into "late" and "non-late testers". Absolute numbers on Y-axis.*

6.1.1.1 Demography of the study-patients

The demographic characteristics of the patients are depicted in *Table 3*. In summary three quarters of the patients were male. Median age at diagnosis was 34 (range 17-91) years. Heterosexual route of transmission dominated (46%), one third were MSM and 13% infected through IDU. The majority (58%), originated from Sweden, 23% from Africa and 9% from Asia (*results not shown*). A total of 44% were infected in Sweden, whereas 17% were born in Sweden and infected abroad and 35% born and infected abroad. For 3% there was no data.

This predominance of males could to a large extent be explained by nearly half of them belonging to the MSM group. However male sex was still predominant among heterosexuals born in Sweden (75 vs 25%), whereas the proportion of women was slightly higher among patients born abroad and infected heterosexually (55 vs 45%). In comparison with just above 60% males, among PLWH at present (*data obtained from InfCare HIV*), this reflects the change in the Swedish HIV-1 epidemic, which is now being dominated by heterosexually infected patients, born abroad. Naturally an additional explanation for the large proportion of males in our study-population could be an assumption that males are more likely to develop AIDS and thus more likely to have been included in this study. However, an analysis of the likelihood of an AIDS diagnosis, in our more recent cohort (paper II and III), shows no such association (*results not shown*).

	"Late" (%)	"Non-Late" (%)	P-value
Absolute number	219 (45)	268 (55)	
Gender			
Female	59 (27)	68 (25)	
Male	160 (73)	200 (75)	
Age			
<40	113 (52)	209 (78)	
≥40	106 (48)	59 (22)	P<0.001
Route of Transmission			
Heterosexual	116 (53)	108 (40)	P<0.01
Men who have Sex with Men	77 (35)	89 (33)	
Intravenous Drug Users	6 (3)	59 (22)	P<0.001
Blodproduct/transfusion	2 (1)	8 (3)	
Unknown	18 (8)	4 (1)	
Origin/Place of Infection			
Infected in Sweden	76 (35)	139 (52)	P<0.001
Borne in Sweden-Infected Abroad	51 (23)	33 (12)	P<0.002
Borne and Infected Abroad	79 (36)	93 (35)	
Unknown	13 (6)	3 (1)	

Table 3. *Characteristics of 'late' and 'non-late' testers diagnosed with AIDS in Sweden 1996–2002.*

6.1.1.2 Characteristics of the late tester

The sex distribution among the late and non-late testers was similar, with approximately 75% males in both groups, on the contrary to other studies where male gender have been associated with a late diagnosis [110, 111].

The proportion of patients aged above 40 years were more than twice as high among the late testers than the non-late (48 vs 22%, $p<0.001$) and age as an important factor associated was at the time also described by others [110, 163, 164]. Among men in the study the late testers were approximately ten years older than the non-late tester, with a median age of 42 and 33 years respectively ($p<0.0001$), whereas there was no significant difference in women, with a median age of 31 years. This could be explained by the female predominance among migrants, whom were younger than those patients born in Sweden, but also more likely to be diagnosed late (*see below*).

In the initial year of the study the majority of late testers were MSM, but then there was a shift with an increasing incidence of those heterosexually infected (*Figure 12*). Overall heterosexual route of transmission was predominant in both late and non-late testers, but more prevalent among the prior (53 vs 40%, $p<0.01$), whereas the proportion of MSM was similar, with approximately one third in each group. A major difference were seen for PWID, which constituted only 3% (6/219) of late testers compared to 22% (59/268) of the non-late $p<0.001$). A similar pattern, with increased risk for heterosexuals compared to PWID to be diagnosed late, was described in Italy [111], but other studies at the time showed conflicting results [110, 162]. Whereas this could indicate a higher perception of risk associated with IDU, both among patients and the caregiver, another explanation naturally could be a disproportionally higher prevalence of disease progression to AIDS, despite a known HIV diagnosis, in this group, as a result of the inferior adherence to treatment and/or retention in care [188]. Overall the highest proportion of late testers was seen in those with an unknown route of transmission, 82% (18/22).

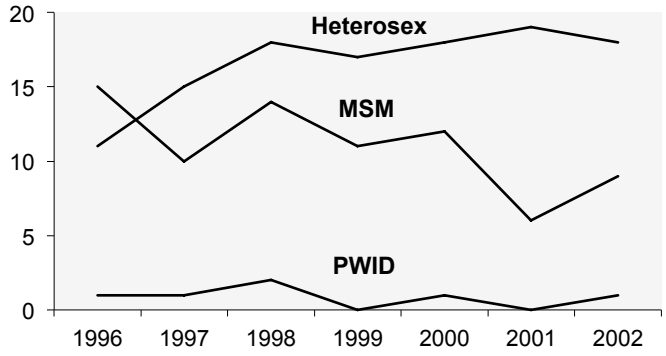

Figure 12. *Late testers in Sweden 1996-2002, by route of transmission. Absolute numbers on Y-axis. (MSM = men who have sex with men, PWID = people who inject drugs).*

Patients infected in Sweden were less likely to be late testers, in comparison with the total of other patients (35 vs. 53%, $p<0.001$). A significant difference also remained when the comparison was restricted only to those born and infected abroad (35 vs. 46%, $p<0.05$) (*results not shown),* and foreign origin as an important factors associated with late HIV-1 diagnosis, described by others [110, 163, 164], thus confirmed. Interestingly, for Swedish born patients infected abroad, predominantly represented by heterosexual men infected in Asia, a significantly increased risk of testing late were seen in comparison with others (61 vs. 42%, $p<0.002$), but also in a comparison including only those patients born and infected abroad (61 vs. 46%, $p<0.05$), indicating a low awareness or ignorance in this group.

Over the study period the proportion of late testers born abroad increased from approximately one quarter in 1996 to more than half in 2002, as a result of a decreasing AIDS incidence among Swedish-borne patients, but also in absolute numbers the late testers born abroad showed an increasing trend (*Figure 13*).

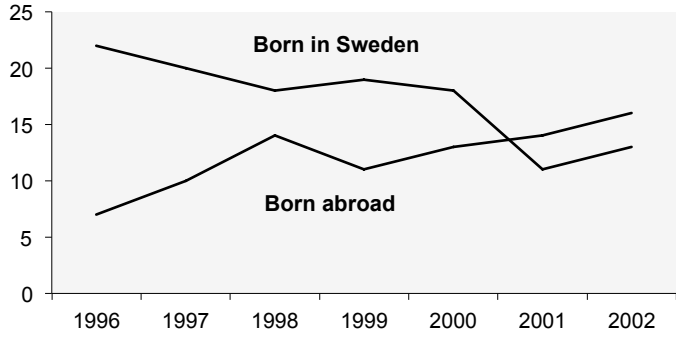

Figure 13. *Late testers in Sweden 1996 -2002 by origin. Absolute numbers on Y-axis.*

6.1.2 A need for further studies

In this study, one of the first national studies on late HIV-1 diagnosis in the era of cART and the first in Sweden, we found evidence confirming that late diagnosis was a remaining problem and that the group without previously known HIV-1 infection represented an increasing part of all cases of AIDS. We found age greater than 40 years, heterosexual route of transmission and foreign origin to be significant risk factors for being a late tester whereas intravenous drug use was associated with a highly significant reduced risk.

In our characterization of the patients with late HIV-1 diagnosis we made a comparison with all patients, whom had developed AIDS, after an early diagnosis during the same period.

Others had made similar comparisons [111, 112] and Castilla [110], whom had published the first national study on late diagnosis post-cART in 2002, inspired us to use AIDS as the denominator. In retrospect it would have been preferable to use all patients diagnosed early as the control group, as we choose to do in paper II-III, to be able to assess the characteristic of a patient with a late, as a contrary to an early, diagnosis.

Additional weaknesses to be discussed are related to the possibility to test anonymously for HIV in Sweden and the fact that patients tested HIV positive are reported by a non-unique code; if a patient had been tested HIV positive earlier, but turned up several years later with an AIDS defining condition, without disclosing the earlier test, we would not have known about it. Likewise, we would not always have known if a migrant with AIDS had had a previous test before immigration. Finally, the national register of HIV and AIDS, used for the study, contained limited data on the date of immigration of migrants, which prevented us to evaluate whether the late diagnosis could simply be explained by the patients being severely immunocompromised and ill already at arrival or if the AIDS diagnosis could be explained by a delay in diagnosis in Sweden.

On the contrary to many countries, where HIV is not a notifiable disease, this study had the strength of being based on a central reporting system, where Swedish physicians were obliged to report both HIV and AIDS, separately. This minimized the risk of missing any cases and also gave reliable figures on the time between HIV and AIDS. The questionnaire, used to collect additional information after the initial reporting, also contributed to fewer mistakes and missing data.

Despite weaknesses in the study two important statements could be made; Late HIV-1 diagnosis was identified as a true problem in Sweden and there was no evidence of a decreasing trend. We could also conclude that the proportion of late testers born abroad was continuously increasing. From an infectious disease control and medical perspective I found it important to study this area further to assess what measures are needed for an earlier identification and care of our patients.

6.2 PAPERS II-III

6.2.1 Patients included

For these studies we included patients with newly diagnosed HIV-1 infections, prospectively identified, directly from the Swedish HIV clinics, rather than from a retrospective register. The regional Ethics committee in Stockholm gave us the permission to use unidentified data, from the InfCare database, for all patients during the study period. Thus all 575 newly diagnosed patients at the twelve study clinics could be included in the study and the representativeness of our cohort evaluated. A comparison with all patients linked to Swedish HIV Care during the study period (n=827), showed no significant differences regarding demography indicating that our data was representative of the country as a whole. (*Figure 8*).

Most study patients, 71%, participated in the sub-study with the questionnaire. Reasons for not participating were; rejection 11%; medical 7%, loss to follow up 6%, and logistic 5%. For Swedish born patients, of whom 78% participated, there was no significant difference regarding demographics between those who participated or not. For migrants, of whom 67% participated, those estimated to be infected in East or SSA were more likely not to take part than those infected in Sweden [OR 3.2 (95% CI: 1.4-7.1); OR 2.9 (95% CI: 1.4-5.9), respectively] (data not shown). However, these groups were still well represented, with just above 60% recruited from each group.

For 98% (n=566) of the patients the first CD4+ T-cell count was within 3 months, for 1% (n=6) within 6 months. All CD4+ T-cell counts were drawn while the patient was still untreated with ARV. For 1% (n=3) it was missing, but due to a recorded PHI (n=1) or AIDS-diagnosis (n=2) all patients in the study could be successfully classified according to "LP-status". Comparing this between patients, participating in the sub-study and not, no significant differences were seen.

Baseline demographics are depicted in Table 1a, Paper II. In summary two thirds of the patients were male. Median age at diagnosis was 40 years. Heterosexual route of transmission dominated (54%), one third was MSM and 5% infected through IDU. About one third originated from Sweden, an additional one third from sub-Saharan Africa and the remaining from all parts of the world but predominantly Thailand. A similar pattern was found for route of transmission, but with patients infected to a little lower extent in the SSA in favor of the Asia and Pacific.

6.2.2 Late Presentation and advanced HIV disease – key problems

A majority, in total 58% (331/575), of all newly HIV-1 diagnosed patients were Late Presenters, of whom two thirds (218/331) already had developed an advanced disease; 56% (123/218) based on CD4+ T-cell count alone and 44% (95/218) with an AIDS-diagnosis.

Another Swedish study [132], conducted by the Swedish Institute of Infectious Disease control, of patients infected in Sweden or born in Sweden and infected abroad, 2003-2010, using so called "BED-tests" indicated that as many as 63% had been infected more than six months at diagnosis. Considering the fact that patients of foreign origin, estimated to be infected abroad, but still living in Sweden before the diagnosis, were not included these results are well in line with ours.

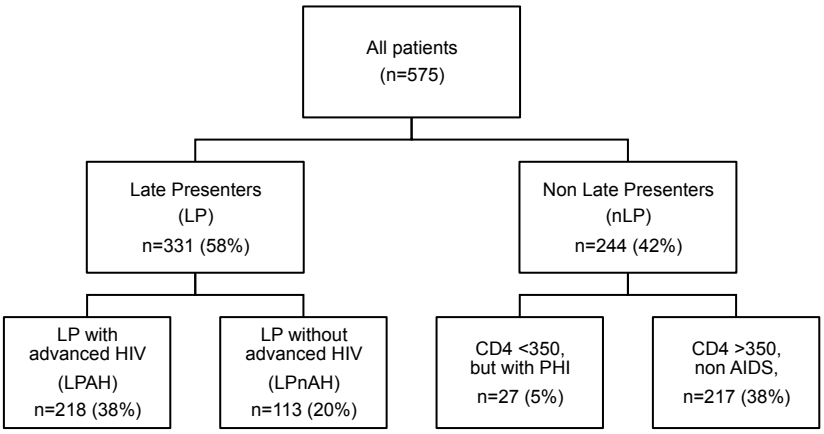

Figure 14. *The distribution of patients in the categories late and non-late presenters of HIV-1 infection in Sweden 2009-2012.*

On the European level also the Collaboration of Observational HIV Epidemiological Research Europe (COHERE) study have reported consistent results with >50% of newly diagnosed patients in Europe fulfilling the definition of Late Presentation and as many as one third with an advanced HIV disease, when presenting for care [1].

Even though there are numerous initiatives and interventions to promote earlier diagnosis there has been limited success. A large meta-analysis of studies evaluating the first CD4+ T-cell count at diagnosis in resource rich countries showed no meaningful improvement since the introduction of HAART in 1996 [189]. Similarly a small decreasing trend of LP in Europe, by 4% yearly, 2000-2010, reported by COHERE [1], has halted with no change over the last years [190]. Also the proportion of persons with advanced disease or AIDS have remained stable [190]. Splitting the data into different transmission groups and regions however shows a tendency of a slight decrease of LP in the North of Europe whereas LP among PWID is becoming more prominent.

In Sweden a recent "post-study" compilation of national data 2009-2014, without exclusion of PHI or those already known to be infected, shows no changing trend in late presentation (*InfCareHIV 20141231* (*Figure 15*). Clearly a lot remains to be done.

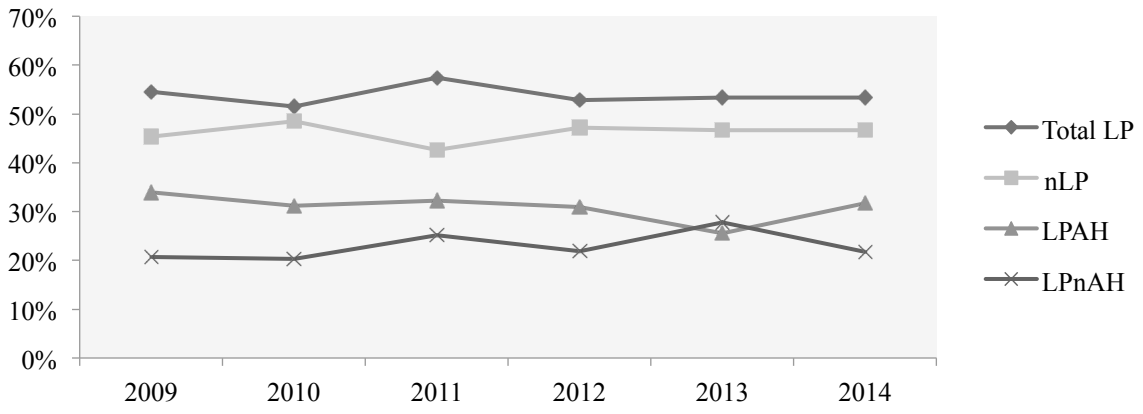

Figure 15. *Proportion with Late Presentation (non advanced/advanced HIV) and non-Late Presentation among patients newly linked to Swedish HIV care 2009-2014.*

6.2.3 Late Presentation is associated with age, origin and country of transmission

At first sight, also confirmed by the analysis on the bi-variate level, the LP were more likely to be female than male (66% vs 53%; p<0.01) and older than the non-LP (mean years: 41 vs 38; p<0.01). The LP also seemed more likely to be heterosexually infected than trough IDU or being a MSM, with proportions of LP of 67, 43 and 40%, respectively (p<0.01). There was also a significant difference according to origin with the proportion of LP ranging from 81 and 74% respectively in patients from *Asia and the Pacific* and the *SSA* regions compared to 43% in patients born in Sweden.

In a multivariable analysis, adjusted for all demographic variables (*Table 2, Paper II*) age, origin and country of transmission remained significant, but no association was found neither for gender nor route of transmission. For advanced HIV a borderline reduced odds were seen for MSM (0.6 (0.3-1.0, p= 0.059) and it is possible that an association with reduced odds would have been identified if we had had a larger study-population. Still 40% of MSM were LP indicating a problem with late diagnosis in all transmission groups, which must be taken seriously.

6.2.3.1 Age

The single factor most associated with late presentation was age, with a continuous increase from OR 1.6 (1.0-2.7) if 31-40 years to 4.0 (2.1-7.6) if >50, compared to those <30 years. A similar, but even stronger, association with age was seen for patients presenting with an advanced HIV disease. Similar results have been presented in other studies suggesting a low perception of risk, both among patients and health care professionals [168, 191-196], whom often assume sexuality to be an insignificant part of an older patients life [197], as one main explanation.

To find age associated with late presentation is not surprising considering that an infected and undiagnosed patient naturally progress in immunodeficiency when they get older. However this is clearly not the entire explanation and there are British studies suggesting that nearly half of HIV-1 positive individuals > 50 years acquire their HIV infection at age 50 and over [198]. An American survey also suggests that three quarters of individuals 57-64 years of age, just above half of those 65-74 years of age and one quarter of those 75-85 years are sexually active [199]. Additionally around 10% of 57-85 year olds have reported a new sexual relationship the last five years [200]. As for HIV and late diagnosis a recent study assessing new HIV-1 diagnosis at autopsy also found age ≥65 years as the strongest correlate for this [201].

The 17% of patients >50 years in our study population represents one of the higher proportion of older patients reported in Europe [202]. Since age is associated with a faster disease progression of HIV [198] a special focus on timely diagnosis and care is needed in this group.

6.2.3.2 Origin and Country of transmission

Apart from age late HIV-1 diagnosis was associated with origin and country of transmission. Increased odds of LP were seen in patients originating from the East [OR 3.5 (95%CI: 1.8-

6.9)] and the SSA [OR 2.7 (1.4-5.5)], compared to those born in Sweden. Additionally there was an independent association also with the country of transmission, with increased odds for those infected in the SSA [OR 2.6 (1.3-5.3)] and for those with an unknown place of HIV acquisition [OR 3.8 (1.6-9.2)].

It is well documented that patients of Asian, black African and South American ethnicity have a higher probability to be diagnosed late [196, 203]. Creating an additional variable, including simultaneously both origin and country of HIV transmission, similar to what we did for paper I, however showed interesting results. A comparison of the patients *born and infected in Sweden* (22%) and those *born abroad and infected in Sweden (*11%)*,* while still adjusting for age and route of transmission, showed no significant difference between the groups, indicating that the ethnicity might just not hold as an explanation. Comparing instead those *born and infected in Sweden* with *migrants reported to be infected abroad* (50%) a higher odds [OR 3.2 (95%CI: 1.9-5.6)] of being a LP were found for the latter group. The patients *born in Sweden and infected abroad* (10%) did not differ significantly from those *infected in Sweden* and the higher probability of a late HIV-1 diagnosis in this group, reported in paper I, thus no longer found.

Even if there was a considerable lower probability of late diagnosis for all patients infected in Sweden and for those Swedish born infected abroad, still as many as 41% of these were LP.

6.2.3.3 Determinants of Late Presentation for patients born in Sweden

Considering the more detailed data collected in the sub-study, age >50 years [OR 7.8 (95%CI: 2.0-31.2)] and unknown country of transmission [OR 15.3 (95%CI: 1.7-141.3)] were associated with being a LP for Swedish born patients. Patients with a psychiatric illness had reduced odds [OR 0.2 (95%CI: 0.1-0.6)], compared to those who had not. Comparing instead patients with advanced HIV disease (LPAH) with non-LP (nLP) the association with age was even more prominent [OR 15.5 (95%CI: 1.7-137.7) if 41–50 years old and 23.6 (95%CI: 2.6-215.8), if >50 years old]. Also the association with unknown country of transmission [OR 12.8 (95%CI: 1.2-138.1)] and with psychiatric illness [OR 0.2 (95%CI: 0.0-0.7), $p<0.05$] remained. Results were adjusted by missed HIV-associated symptoms. (*Table 3a, Paper II*).

The finding of a lower probability of late diagnosis among patients with psychiatric disease has, to our knowledge, not been described before. Possible explanations could be the tendency among physicians to primarily offer tests to patients perceived to be at high risk and also to those most likely to accept a test [204] in combination with more frequent health care contacts in this group.

6.2.3.4 Determinants of late presentation for migrants

For migrants participating in the sub-study age was also associated with being a LP, with increasing odds by each age category [OR 2.2 (95%CI: 1.1-4.6) if 31–40 years to an OR 4.2 (95%CI: 1.3-14.3) if >50 years, compared to <30]. An increased probability to be a LP was also seen for those patients who had spent less than two months in Sweden at diagnosis [OR

3.2 (95%CI: 1.1-9.4)] in comparison with those who had lived in Sweden for five years or more. The model was adjusted for country of origin and country of transmission.

Comparing instead LPAH with nLP and adjusting for psychiatric illness, similar, but more pronounced associations were seen for age >30 years and time spent in Sweden, both for less than two months [OR 3.8 (95%CI: 1.1-12.2)] and 1–5 years [OR 2.7 (1.0-7.0)], compared to > five years. Also for patients with country of transmission stated as SSA [OR 3.8 (95%CI: 1.4-10.4)] or the East [OR 3.6 (95%CI: 1.2-10.9)], compared to Sweden, and for those with previously missed HIV-associated symptoms [OR 3.4 (95%CI: 1.4-8.0)], compared to those without, an increased probability of presenting late was seen. (*Table 3b, Paper II*).

6.2.4 Missed opportunities

In our studies we investigated three kinds of missed opportunities: ***missed opportunity to test at immigration***; the "*doctors's delay*": ***missed at presentation*** and "*patient's delay*": ***symptoms neglected by the patient***. Altogether 65% of the patients, whom had participated in the sub-study with the questionnaire (n=409), had at least one missed opportunity.

6.2.4.1 Missed opportunity at immigration

The greatest of missed opportunities was the *missed opportunity to test at immigration*, which was seen in two thirds (168/253; 66%) of all migrants. Of these 54% were born in the SSA and 28% in the East (predominantly Thailand), i.e. regions with a high HIV-1 prevalence (*data not shown*). Additionally we found that half (56%) of all migrants had lived more than one year in Sweden at diagnosis. Interestingly only one patient, of those offered a test at immigration, reported to have declined the test. (*Data not shown*).

The above could partly be explained by the fact that health examinations in Sweden predominantly have been offered to refugees and not those immigrated due to family connections or work, but there have also been problems with the structure and coordination between county councils and reports from the Public Health Agency of Sweden have suggested that only 40% of those entitled to health screening actually have been reached [205].

For further results about the association between a missed opportunity at immigration and late HIV-1 diagnosis in the migrant population, *see 6.2.7 below*.

6.2.4.2 Missed AIDS- and HIV-associated conditions at presentation for care

Details on the missed presentations are depicted in Figure 1a-c, Paper III. In total 27% (112/409) of patients had presented for health care services with at least one AIDS- and/or HIV-associated condition, without that an HIV-test had been performed. The more serious, *AIDS defining events* (n=31), were seen in 6% (23/409), predominantly in primary care (55%). Most common were the unspecific wasting syndrome (n=14), followed by candida of the oesophagus (n=9), but there were also odd numbers of the more HIV specific Kaposi sarcoma, PCP and PML. One quarter (104/409) of patients (26% of LP; 25% of nLP) had presented at least once, with one or more *HIV associated conditions* (n=162). Whereas

seborrhoea (n=19) and oral candida (n=18) were the most common conditions in LP, another STI (n=22) and PHI-like illness (n=18) dominated among the nLP. Just like for missed AIDS-defining conditions most missed presentations were seen in primary care (58%), but a substantial proportion were also in STI/skin clinics (16%).

Other studies, from countries like Denmark, Italy, the UK and Scotland [167, 168, 206, 207], have reported missed opportunities to diagnose HIV at similar levels as ours. In a recent Dutch study comparing newly diagnosed with HIV-negative controls, 62% had visited their GP the prior year, compared to 39% of controls and as many as 59% had presented with an indicator condition during the five previous years, compared to 7% of the controls, [208]. Another, French, study reported that 82% of those newly diagnosed who had presented for care the three previous years had not been offered testing and neither had a majority of MSM, despite reporting their sexual orientation [179].

6.2.4.3 Patients missed at presenting for care

In a multivariable regression model, adjusted for route of transmission, patients from the East or SSA had reduced odds of missed presentations (OR 0.4; 95% CI: 0.2-0.8, $p<0.05$; OR 0.3; 95% CI: 0.2-0.6, $p<0.001$, respectively) compared to patients born in Sweden. A borderline reduced odds was also seen for patients from *Other countries* (Western Europe, North and Latin America, Caribbean, North Africa, Israel, Middle East) indicating that being Swedish born was the most important factor associated with a missed HIV diagnosis while presenting for care.

It is well known that HIV-tests are primarily offered to those perceived to be at high risk [204] and the above consequently indicates a good adherence to one of the most prominent epidemiological risk factors, foreign origin [167]. Somewhat surprising we did not find any association with route of transmission, where a reduced risk to be missed for MSM could have been anticipated. Probably this could be explained by sexual orientation not being as obvious to the physician as an origin from epidemic area and seldom asked for, but as mentioned above MSM have also been reported to be missed despite reporting their sexual orientation [179].

6.2.4.4 Missed presentations and late HIV-1 diagnosis

In our study the probability to have an earlier missed presentation was twice as high among LP with advanced HIV compared to non-LP (OR 2.0; 95% CI 1.2-3.1, $p<0.01$) and consequently there was also a significantly lower median CD4+ T-cell count at diagnosis for those who had a missed presentation; 150 versus 290 cells/mm^3, p=0.0001 (*Figure 16*).

If testing, on the other hand, is performed at presentations with indicator diseases a reduction in LP, with as much as 50%, have been described in those tested compared to those not [207]. With one quarter of nLP reporting missed presentations in our study it is evident that great improvements could be possible. Furthermore our study-design, where we restricted the medical history to the previous three years and to a predefined list of HIV-associated conditions, most likely made us underestimate the extent of the problem indicating that even greater accomplishments could be made.

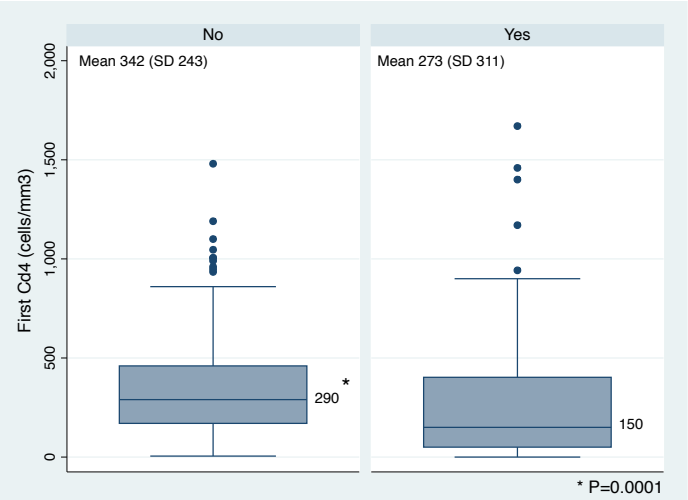

Figure 16. *CD4 count at diagnosis in patients without (No) and with (Yes) a missed HIV test when presenting for health care. *Wilcoxon test shows a significant difference.*

It is obvious that the predominant strategy of risk factor based HIV testing, traditionally used in Europe, fails to identify many patients. Most of the HIV-associated conditions used in our study has also been evaluated in the HIDES-study and proved to exceed the 0.1% needed to reach cost effectiveness of general screening [166]. However, despite demonstrated as appropriate triggers for HIV testing a lot remains to be done to make the use feasible in Europe, were testing rates in well established indicator conditions were still low in 2013 [209].

6.2.4.5 Patient´s neglect

Sixty-five patients (16%) in our study had a history of 92 neglected AIDS- and/or HIV - associated symptoms the three years prior to their diagnosis. For LP, the most commonly neglected symptoms were weight loss (19%) and fatigue (15%). Among nLP a "PHI like syndrome" was most common, definitively described by 38% and possibly by additional 35% (data not shown).

Just as for the missed presentations, at seeking health care, the probability of neglecting symptoms was lower among patients originating from the East (OR 0.3; 95% CI: 0.1-1.0, $p<0.05$) and the SSA (OR 0.4; 95% CI: 0.2-0.8, $p<0.05$) compared to patients born in Sweden. A lower probability of neglecting symptoms was also seen for MSM (OR 0.5; 95% CI: 0.2-1.0, $p<0.05$) compared to those heterosexually infected.

As far as we know ours is the only study describing the patients who experience symptoms without seeking health care, but it is well in line with other studies describing a low perception of risk for HIV as one of the greatest barriers to testing [175, 180]. Even though our use of an open question, allowed for all kinds of HIV-associated symptoms to be reported, the three-year time frame most likely resulted in an underestimation of symptoms. However the low number, in comparison with the missed presentations for care could indicate that barriers to testing is larger on the provider level, also described by others [204].

6.2.5 The initiator of HIV-testing

In our study a health care provider initiated 75% of all HIV-tests that lead to a diagnosis, whereas the patient was the initiator in 25%.

Of the provider initiated tests 44% were due to symptoms and 56% due to different kinds of screening (screening in high prevalent groups 31%; contact tracing 15%; maternal health/blood donors 10%). The median CD4+ T-cell count at diagnosis were significantly lower count for those patients tested due to symptoms compared to those whom had been the subjects of screening (110 vs. 300 cells/mm^3), irrespective of PHI and other early symptoms also being included in this group. The highest CD4, however not significantly different from those diagnosed through screening, was seen for those tested on their own initiative, 375 cells/mm^3 (*Figure 17*).

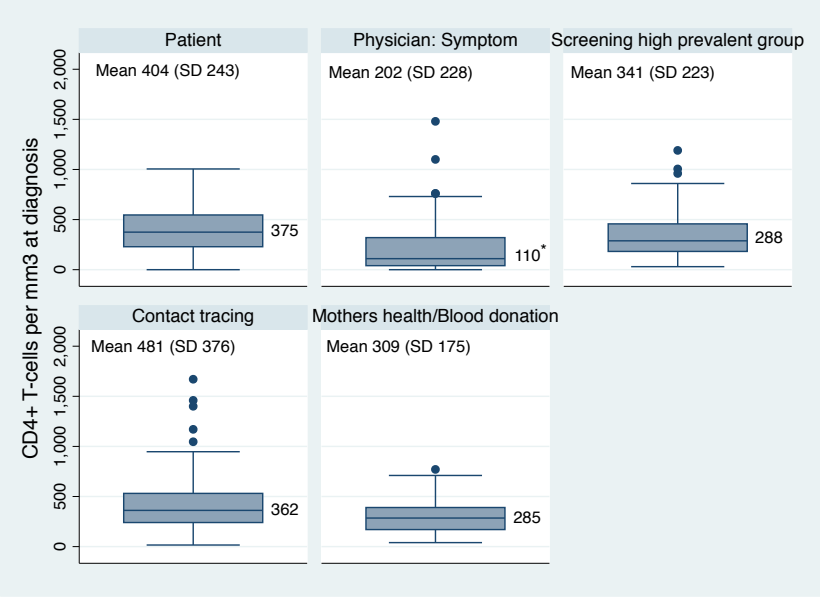

Figure 17. *CD4+ T-cell count at diagnosis, divided by the initiator/reason of the HIV-test. The Kruskal Wallis test shows a significant difference comparing group 2 with group 1, 3 and 4.*

A difference was seen for gender with men more likely to take the initiative to test (33% vs. 14% in women), whereas women were more likely to be diagnosed through screening (57% vs. 33% in men), p<0.001. This difference could to a large extent be explained by MSM often tested on their own initiative (33% vs. 19% of heterosexuals, p<0.001) and many women tested due to maternal screening (18%). The proportion of patients tested due to symptoms increased with age (from 28% among those <50 years to 56% among those >50 years, p<0.001). Whereas most of the Swedish born patients were diagnosed due to symptoms (39%) and almost as many due to testing on their own initiative, the majority of patients from the SSA (65%) were diagnosed through screening and only 12% through an own initiative to test. For patients from the East the initiator of the test was equally distributed.

6.2.5.1 Patients whom take initiative to test for HIV

In a multivariable analysis assessing the patient as the initiator of the HIV-test patients infected in the East, with a history of drug use or a previous negative HIV test (predominantly seen among MSM) were more likely to take the initiative, whereas patients older than 50 years and those with a history of a previous missed presentation had reduced odds, p<0.05.

This could indicate both an increased awareness in the prior groups, but also better availability to test in terms of STI clinics and drug addiction treatment centres [104].

The low probability to initiate a test among individuals with a missed presentation is important and indicates that a missed presentation could have further implications than just that of one missed opportunity. Since many patients believe that a HIV-test is routinely performed, whenever they have their blood drawn [210], or as they often have stated to me: "It should have shown in the blood somehow, should it not?", a missed presentation could give the patient a false sense of not being seriously ill and thus possibly delay further health care contacts.

6.2.6 Increasing HIV testing

Serious attempts to increase HIV testing have been made throughout Europe. In the UK the national HIV guidelines in 2008 recommended HIV testing to be considered both in general practice and at all general medical admissions in regions with a HIV-prevalence exceeding two per 1000 [211]. The following year a one-time voluntary HIV screening test was recommended for the whole population in France [170]. However the adherence to these guidelines have proved to be difficult, both due to general feasibility and to economical issues [179, 212], even though there are a few good examples [213-215].

Our studies indicate that both awareness of HIV and availability to test are important factors to enhance earlier diagnosis. We can also conclude that the screening activities performed in Sweden today catch patients at an earlier stage of disease than if the patients are diagnosed when presenting for health care with symptoms. Whereas it has been described that physicians are more likely, and comfortable, to offer a test in a screening setting than due to an indicator condition the opposite has proved to be true for patients, who are more likely to accept testing if being symptomatic [204]. To launch the Indicator Conditions, elaborated by the HIV in Europe initiative [165], as triggers for "screening" thus seems to be a possible way forward. This kind of opt-out approach, when the patient is informed that a HIV test will be included as one in a standard batch of tests and that they specifically need to state if they do not want to be tested has been associated with higher rates of testing both compared to opt in approaches and surprisingly also mandatory testing [104].

That routine screening, without singling out specific patients, is efficient has been shown, not the least in antenatal screening where both uptake and acceptance are very high [165]. Hopefully an implementation of routine screening at certain conditions will be more feasible to use within Swedish health care, than the predominantly used "risk-factor" based testing, even though screening in high prevalent groups naturally should not be forgotten.

6.2.7 HIV-1 diagnosis in migrants

Even though individuals from high prevalence countries, predominantly from the SSA, constitute a substantial proportion of the HIV-1 positive individuals in Europe [88] and as many as one third of newly HIV-1 diagnosed in the EU/EEA region are born abroad, information and data on HIV prevalence, incidence and post arrival acquisition of HIV among migrants are scarce [216].

In our study of newly HIV-1 diagnosed patients (papers II-III) almost two thirds were born abroad, which represents one of the higher proportions described in Europe [168, 192, 193, 195, 196]. We also concluded that this group represents a particularly vulnerable group, not only to HIV itself [217], but more specifically so to late presentation (65%) and advanced HIV disease (43%), also reported by others [168, 192-196, 218, 219]. In total almost three quarters of all LP (74%), and LPAH (73%), were born abroad.

Whereas 17% of the migrants were reported to have acquired their HIV-1 infection in Sweden, the vast majority were reported to have been infected abroad and it could easily be claimed that late presentation is not a major national problem, but rather explained by patients being severely ill upon arrival and possibly already aware of their diagnosis. To further investigate this we modelled the time resident in Sweden, for those migrants estimated to be infected abroad, with the probability of Late Presentation and Advanced HIV (*6.2.7.1*) as well as with the reason for testing (symptoms/patient's initiative/screening) (*6.2.7.2*) up until five years after arrival.

6.2.7.1 Time in Sweden and the probability of a late HIV-1 diagnosis

For those patients tested within two months of arrival the probability to be a LP or LPAH was 80% and 50% respectively. The proportion of LP, particularly the LPAH, then decreased to further increase again from four months and then continuously increase until three years after arrival. A similar, but even more pronounced pattern, were seen analysing only the patients from SSA, who represented the majority of patients. For patients from the Asia and Pacific region, the probability of being a LP at time of arrival was 70% with a small increase during five years after immigration and a doubling of the probability to be a LPAH (from 40% to 80%) during the first three years, which then became stable.

Interestingly an overall increase in nLP was seen after three years. Whether this was due to a "survival of the fittest", so called slow progressors, or could be explained by infections actually acquired after immigration is uncertain. Against the prior speaks the fact that this tendency was seen in patients from the SSA and not from the Asia and Pacific region. To analyse the latter we took the initiative to paper IV, *see 6.3 below.* (*Figure 18.A*).

6.2.7.2 Time in Sweden and the reason for HIV testing

For all migrants screening, primarily through health examinations, dominated as the reason for HIV testing the first year after arrival whereas the probability to be tested due to symptoms continuously increased from very low levels to 50% two and a half years after arrival and then remained stable. Overall the patient's initiative to testing was low, but more common at arrival (25%) and at two years after arrival. This lack of initiative may reflect difficulties with access to health care, lower socioeconomic status or increased stigma in these groups [103]. It also dismisses speculations of immigration due to HIV-1 related illness per se. Also, a finding that drug resistance in major as well as in minor quasipecies can be detected only in a minority of patients at diagnosis, suggests that few of the migrants have been treated before [220].

More specifically, for patients from the SSA >80% of those diagnosed within the first months after arrival were identified through screening. The detection rate through the screening

procedure then continuously decreased to 40% after three years, while testing due to symptoms instead became dominating. After three years, screening once again became the main testing cause. Overall the patient initiated testing was low, around 20%.

Among patients from the Asia and Pacific region half of those tested the first months after arrival took the initiative to test, but there was then a decline to a very low percentage after three years. A similar patter was seen for screening, whereas the probability to be diagnosed because of HIV/AIDS-related symptoms increased from less than 20% to almost 100% after 40 months. (*Figure 18.B*).

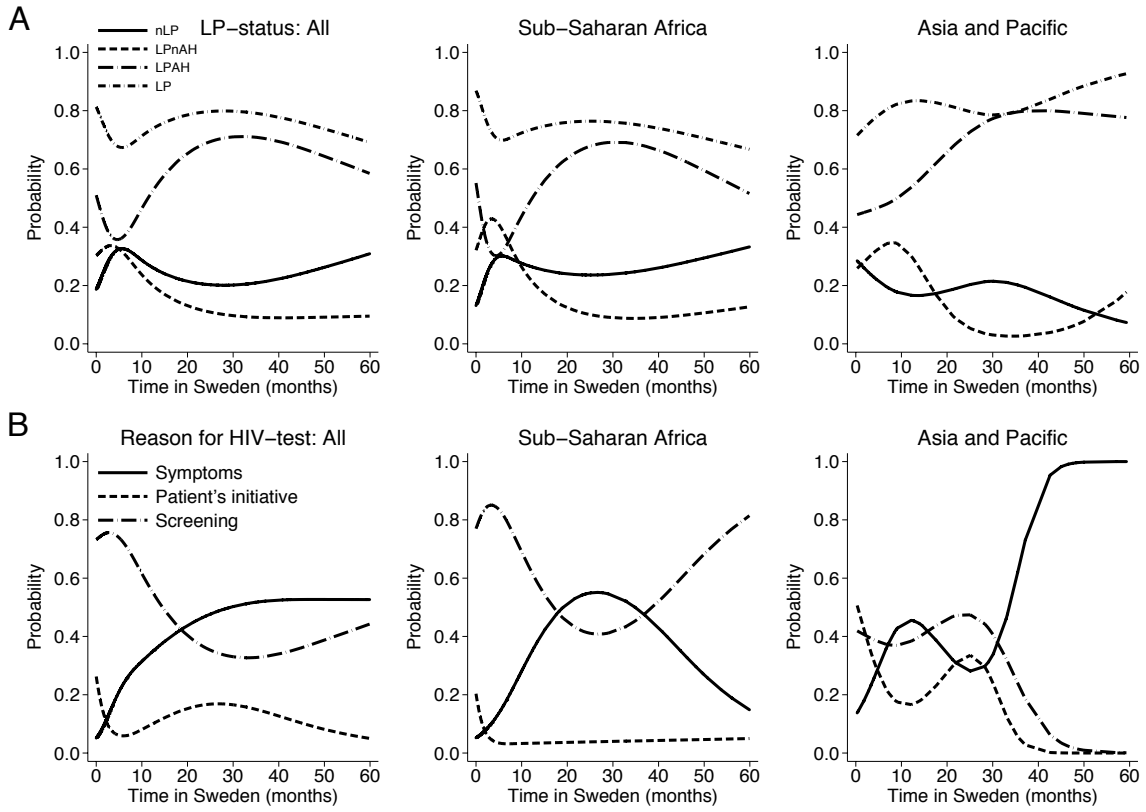

Figure 18. *Migrants infected outside Sweden: Proportions of late presenters (LP) with non-advanced (LPnAH) or advanced (LPAH) disease and non-LP [A] and reason for HIV-testing [B], in relation to time in Sweden, at diagnosis.*

6.3 PAPER IV

The evidence from Paper II, that half of all migrants had lived >1 year in the country at diagnosis and that two thirds had not been tested at immigration, implied that there is a breeding ground for HIV-1 transmission in Sweden and that the domestic infections might be higher than reported. Our modelling of the time resident in Sweden, that showed a trend towards a higher proportion with early diagnosis three years after arrival, for those migrants estimated to be infected abroad [221], was also well in line with this possibility. In addition, a sequence analysis of circulating HIV-1 strains, among patients reported to be infected in Sweden, have shown an increasing proportion of non-B subtypes and new recombinant forms, in favour of the HIV-1 subtype B, which has dominated the epidemic in Europe from the beginning [28]. With the predominance of migrants in Paper II-III in mind and to further assess whether the number of domestically acquired infections is underestimated we applied the CD4+ T-cell decline trajectory model (*see 5.2*) to the Swedish migrant cohort.

6.3.1 Patients included

A total of 1244 patients were included. Baseline characteristics are depicted in Table 1, Paper IV. In summary mean age at diagnosis was 36 years and 55% were females. 72% were heterosexually infected and 13% were MSM. Patients were predominantly born in Africa (63%), 10% in Europe and the remaining in other parts of the world. Median CD4+ T-cell count at diagnosis was low, 240 cells/mm^3. After excluding patients resident less than six months in Sweden in a sensitivity analysis, similar characteristics were found for the remaining 655 patients, with the exception that the proportion born in Africa decreased from 63% to 54% while those born in countries other than Africa and Europe increased from 27% to 35% (data not shown).

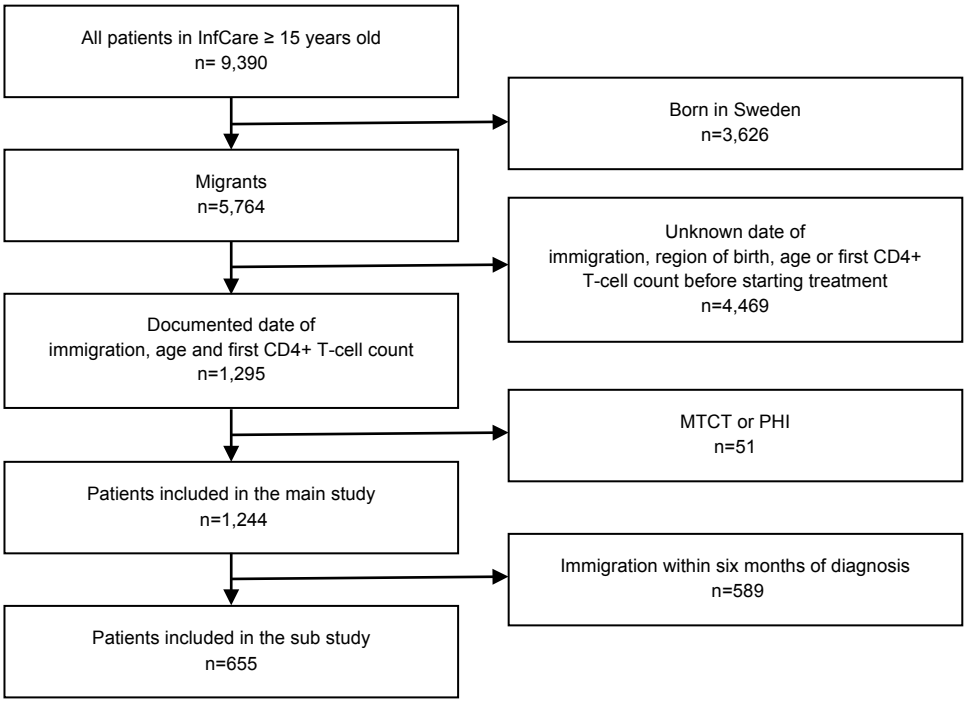

Figure 19. *Flowchart for inclusion of patients in Paper IV.*

6.3.2 The CD4+ T-cell decline trajectory model suggests a higher rate of HIV-1 acquisition post immigration

By applying the CD4+ T-cell trajectory model to our migrant cohort, with a known date of immigration, we received estimates of the time of seroconversion, which we compared to the reported date of immigration in order to estimate whether the patient was infected after arrival to Sweden or not. In total the model estimated 17% to be domestically infected and 74% infected outside, while the corresponding figures registered in InfCare was 11% and 89% respectively. For 8% of the patients the model estimate was uncertain (*Table 4)*.

The estimates of the model and the doctors, documented in InfCare HIV, were concordant for the majority of patients (81%)*, but in 11% they disagreed**. More commonly this was due to the model estimating the patients to be infected after arrival to Sweden while the patient was reported by the doctor to be infected abroad, than vice versa (9.4 vs 1.2%).

Doctor´s estimate n (row %)	Algorithm estimate			Total n (col %)
	Sweden	Outside Sweden	Uncertain	
Sweden	100 (8.0)*	15 (1.2)**	18 (1.5)	133 (10.7)
Outside Sweden	117 (9.4)**	909 (73.1)*	85 (6.8)	1,111 (89.3)
Total	217 (17.4)	924 (74.3)	103 (8.3)	1,244 (100)

Table 4: *Doctor and algorithm estimates of probable country of infection among 1,244 migrants aged 15 or above, without MTCT or PHI, diagnosed with HIV-1 in Sweden with known year of arrival.*

To further assess which of the estimates were most likely to deliver accurate results we performed phylogenetic analysis on viral sequences from the patients of whom the estimates were discordant. Even though there were some weaknesses associated with this analysis, which were performed anonymously on 70% of the patients who had discordant estimates, the phylogenetic analysis indicated a higher concordance with the model estimate than with the estimate of the clinical reports (30% vs. 17%).

In the sensitivity-analysis (excluding patients resident less than six months in Sweden at diagnosis), the model and the doctor's estimate of infection in Sweden increased to 32 and 20% respectively, whereas the uncertainty for the model estimate rose to 14% (data not shown). The majority (93%) of all patients with a discrepancy between the model and doctor's estimates remained and the results of the phylogenetic analysis still favoured that of the model (28 vs. 18%).

6.3.3 Determinants of the patients with conflicting estimates of the country of HIV-1 acquisition

To investigate socio-demographic factors associated with a discrepancy between the doctor's reported country of HIV-1 acquisition and the model estimates we performed two multinomial regression models. The first model, assessing the discrepancy *doctor out/algorithm in* instead of agreement, found that the probability of discordance increased

with age [OR 8.1 (95% CI: 1.8-37.2), if 35-44 years, OR 13.2 (2.7-64.3) if 45-54 years and OR 15.8 (3.0-82.5) if >55 years; p<0.01), compared to those 15-24 years old] and higher CD4+ T-cell count at diagnosis [OR 7.1 (3.6-14.1), if 350-499 cells/mm^3 and OR 9.4 (4.8-18.5) if ≥ 500 cells/ mm^3; p<0.001), compared to less than 200 cells/mm^3]. For PWID there was a decreased likelihood of discordance [OR 0.1 (0.0-0.6); p<0.05), compared to patients heterosexually infected], just like for those with a later the year of arrival [OR 0.4 (0.2-0.7), if 2000-2004, OR 0.1 (0.1-0.3) if 2005-2009 and 0.0 (0.0-0.0), if ≥2009; p<0.005), compared to those that arrived before the year 2000]. There was no association with gender or region of birth, but the latter were kept in the model and adjusted for.

The second model, assessing the discrepancy *doctor in/algorithm out*, included only 15 patients and no significance was found. In the sensitivity-analysis, the determinants were equivalent to the above.

6.3.4 The CD4+ T-cell decline trajectory model – a valuable tool in HIV-1 surveillance

Of all migrants registered in the InfCare database 12% are reported to be infected in Sweden. In our recent studies, Paper II-III, assessing only the newly diagnosed patients, the proportion raised to 17%. Further assessment, by application of the CD4+ T-cell trajectory model to Swedish data, indicates that these estimates, based on clinically reported country of transmission, are likely to be underestimations and highlights the difficulty in estimating the country of HIV acquisition, also described by others [89, 90, 96].

The relatively high, 81%, concordance between the model and the doctor's estimates and the additional phylogenetic analysis, favouring the CD4 model, indicate that the model is a well functioning tool and produces reliable estimates. As a contrary to physicians, who might be biased to assume a patient from a high prevalent country to be infected in their country of origin [90] and to have been infected for a longer duration than is actually true, due to a natural lower CD4+ T-cell count [181, 182, 222, 223], the CD4 model accounts for this and has the advantage to produce more objective estimates.

The CD4 model only estimates whether a patient has become infected before or after arrival to a country, not the geographical place of transmission. Thus, it does not account for the possibilities of travels abroad after immigration. However, since the model estimate is the time of seroconversion rather than the country of HIV acquisition, it is possible that for some patients both estimates could be right; that is when the patient got infected after immigration (model's estimate), but while going abroad (doctor's estimate). The high proportion (52%) of patients with a discrepancy, where the phylogenetic analysis did support neither of the estimates, and the higher probability to have a discrepancy at higher CD4+ T-cell counts (that is a more recent infection) are also well in line with this.

In conclusion our analysis showed that the CD4 method has a good precision in estimating the time of HIV acquisition and confirms the hypothesis that a higher proportion becomes infected after arrival than previously suggested. Independent on whether the infection is acquired in Sweden or while going abroad, after immigration, it is important to further emphasize primary preventive measures among migrants who have established themselves in their new home country.

7 GENERAL SUMMARY AND DISCUSSION

In **Paper I**, which was one of the first national studies on late HIV-1 diagnosis after the introduction of cART, we found evidence of an increasing proportion of patients with simultaneous HIV/AIDS diagnosis in Sweden, including a growing number of migrants in this group, based on retrospective data.

For **Papers II-III** instead, we consecutively recruited newly HIV-1 diagnosed patients from clinics throughout the country and merged InfCare data with information in a questionnaire, obtained through interviews and reviews of hospital records. In order to allow comparisons across Europe we used the new European consensus definition of LP [2]: presenting with CD4+ cells <350/mm^3 +/- AIDS. This definition has been criticized to overestimate the prevalence of LP [224], particularly in MSM, due to the frequent occurrence of low CD4 T-cells during PHI. Therefore, as the first study adjusting for this, we defined all patients with PHI as non-LP.

The majority (58%) of all patients were LP, most commonly in migrants but also in Swedish born. Notably, half of the migrants had lived in Sweden for >1 year at diagnosis and two thirds had a missed opportunity at immigration. Assessment of the time resident in Sweden at diagnosis, the stage of disease and the reason for HIV-testing, for those infected abroad, highlighted the importance of provider initiated testing and clearly suggested that early and more prevalent health examinations could decrease HIV related morbidity and mortality. That this is feasible and that the universal HIV screening can be well accepted also in migrants from low prevalent countries has recently been shown [225].

Many patients had the opportunity to be diagnosed earlier, either having symptoms presenting for care without being tested (27%) or experiencing symptoms without seeking a doctor (16%). My interpretation of our results is that although there are missed opportunities to test many migrants at immigration, the “HIV awareness”, both in health care and among individuals, are higher for those belonging to the classical epidemiological risk-groups, especially when symptoms evolve. On the contrary to patients with a “risk-behaviour”, who are more likely to initiate a test themselves, often at an early stage, many migrants do not initiate the test, but seek healthcare, when becoming ill.

In my thesis I show the importance of HIV testing not only in those with a perceived risk but also in everyone with symptoms indicative of HIV. By increased screening at immigration and implementation of indicator guided testing, earlier diagnosis and timely ART would be possible.

Paper II-III had a high coverage rate (70%) of all newly diagnosed patients in the country, including a substantial proportion of migrants, often not well represented in other studies. In contrast to most studies on LP, where CD4+ T-cell counts often are missing, all our patients could be characterized according to the European consensus definition [2]. Another potential problem is the risk of excluding patients with the most severe disease. Since we allowed patients to be included up to 6 months after diagnosis most of them could be included and a comparison between those participating in the questionnaire with those, who did not, showed no differences regarding LP-status (nLP, LPnAH and LPAH). Well aware that collecting

unbiased information from questionnaires is a complex matter, we adjusted for this. The questions were based on an extensive literature review and involvement of experts in related fields. The physician filled in the answers, in the presence of the patient and if needed, an interpreter was used. In order to minimize recall bias the history of missed diagnosis was limited to three years, which increased the reliability of the data, but, most likely, made us underestimate the extent of the problem.

For **Paper IV**, with the predominance of migrants in Papers II-III and reports on the risk of underestimating HIV-1 acquisition after arrival to the new country in mind, we decided to assess whether this would be true also in Sweden. By applying a CD4+ T-cell decline trajectory model to 1244 migrants, with a recorded date of immigration, we produced estimates of 17% infected in Sweden compared to only 11% clinically reported. Even though just above one fifth of all known HIV-1 positive migrants in Sweden were included, due to a high proportion with missing date of immigration, the model could still be evaluated and the risk of underestimating domestic infections among migrants confirmed. However, to truly estimate the number of infections post-immigration further analysis is needed and planned for.

Considering the fact that a substantial proportion of migrants acquire their HIV-1 infection after arrival to Sweden primary prevention adapted for different migrant groups, needs to be strengthened in the national prevention program.

In summary, in order to enhance timely identification and care, HIV testing should be offered on a broader basis. Since cost effectiveness have been demonstrated at a HIV prevalence of 0.1 % [166] this should be feasible. An increased screening in a wider range of settings would also be a valuable step in normalizing the HIV screening test and could, as a consequence, hopefully contribute to the process of reducing stigma and thereby serve a facilitator for even more testing [180].

8 CONCLUSIONS

- ***Late diagnosis is a key problem in the Swedish HIV-1 epidemic***
  - More than half of the patients are diagnosed late.
  - There are no signs of a decreasing trend 2009-2014.

- ***The majority of the Late Presenters can be diagnosed earlier with a more efficent health care system***
  - Two thirds of migrants were not offered HIV testing at immigration.
  - Half of the migrants had lived > one year in Sweden at diagnosis.
  - One quarter of all newly HIV-1 diagnosed patients had presented for health care with typical HIV- and AIDS-associated conditions without being HIV tested.
  - Almost one fifth of all patients had had HIV- and AIDS-associated symptoms without seeking health care.

- ***Specific factors increase the risk of late diagnosis***
  - Older age and foreign origin are the most important factors.
    However, late diagnosis is found among all patient categories.
  - Patients without epidemiological indicators of HIV are more likely to have a history of missed presentations, to neglect symptoms and are less prone to take an initiative to test for HIV.

- ***The proportion of migrants infected after arrival is underestimated***
  - A CD4+ T-cell trajectory model gives a higher estimate than the physician´s interview.

9 SAMMANFATTNING PÅ SVENSKA

Trots att effektiv behandling mot HIV har funnits i närmare två decennier är HIV/AIDS fortfarande en av de främsta dödsorsakerna i världen. I Europa uppskattas en tredjedel av de individer som lever med HIV vara ovetande om sin diagnos och hälften diagnosticeras sent med konsekvenser i form av ökad sjuklighet, dödlighet, smittspridning och höga kostnader för sjukvård. Syftet med denna avhandling var att analysera omfattningen av sent diagnosticerad HIV-1 infektion i Sverige och fastställa vilka som har störst risk att diagnosticeras sent och varför.

I **arbete I** utförde vi en registerstudie baserad på samtliga patienter rapporterade med AIDS (n=487) till Smittskydds Institutet (SMI) 1996-2002, där jag kunde konstatera att patienter med sen diagnos (här definierade som samtidig HIV/AIDS) utgjorde en ökande andel av individer med AIDS i Sverige. Heterosexuellt infekterade, individer > 40 år och utlandsfödda hade alla högre sannolikhet för att diagnosticeras sent.

I **arbete II** utförde vi en nationell tvärsnitts- och kohort-studie, där vi löpande inkluderade samtliga patienter med nydiagnostiserad HIV-1 infektion vid 12 svenska kliniker. Data inhämtades från det nationella kvalitetsregistret InfCare HIV (n=575) och ett för ändamålet utarbetat frågeformulär (n=409). Sen diagnos definierades som *Late Presentation* (CD4+ T-celler < 350/mm^3 +/- AIDS), vilket uppfylldes av 58%. Ålder (med ökad sannolikhet i och med stigande ålder) samt icke-svenskt ursprung, vilket sågs hos 65%, var starkt associerade med sen diagnos. För utlandsfödda, infekterade i Sverige, sågs emellertid ingen ökad risk jämfört med svenskfödda. Hälften av migranterna hade levt mer än ett år i Sverige vid diagnos och två tredjedelar hade en missad möjlighet att diagnosticeras i anslutning till ankomst. Jag kunde även konstatera att en fjärdedel av samtliga patienter hade missade möjligheter inom svensk sjukvård, då de sökt för HIV- och AIDS-relaterade symtom utan att erbjudas testning samt att 16% negligerat självupplevda symtom.

I **arbete III** analyserade vi vidare de missade möjligheter som sågs i samband med att patienterna sökt vård för HIV- respektive AIDS-associerade symtom samt själva negligerat sina symtom. Vi analyserade även initiativtagaren till testet som slutligen ledde till diagnos. Här kunde jag konstatera att migranter är mindre benägna såväl att ”missas” då de söker vård som att negligera egna symtom, jämfört med svenskfödda. Även individer som identifierade sig som män som har sex med män (MSM) var mindre benägna att negligera sina symtom än heterosexuellt infekterade. Individer som använt droger, testats tidigare (ff a MSM) samt de som infekterats utomlands var mer benägna att ta initiativ till testning, emedan individer >50 år samt de som tidigare ”missats” av sjukvården hade en minskad sannolikhet för detta.

Övervikten av migranter i arbete II-III, och resultat indikerande att andelen som infekterats efter ankomst till Sverige kan vara underskattad, fick mig att vilja undersöka detta vidare. I **arbete IV** applicerade vi en modell, baserad på nedgång i CD4+ T-cells-värde, på den svenska migrant kohorten för att kunna jämföra ett modell-estimerat värde på smittland med vad som rapporterats kliniskt. Totalt inkluderade vi 1244 patienter varav modellen uppskattade att 17% infekterats efter immigration emedan det kliniska estimatet var 11%. För de patienter där det förekom en skillnad mellan estimaten utförde vi fylogenetiska analyser, vilka påvisade en högre överensstämmelse med modell-estimaten (30 vs 17%).

Sammanfattningsvis kan jag konkludera att en stor andel av HIV-1 infekterade patienter i Sverige diagnosticeras sent, men att vi har många möjligheter att förbättra detta. Aktiviteter för att öka den allmänna medvetenheten om HIV, kontinuerligt främjande och normalisering av HIV-testning, utbildning av vårdpersonal och vidare implementering kring begreppet indikatorsjukdomar liksom en utvidgad testning och primärprevention riktad mot våra migranter är samtligt viktiga steg framåt.

10 ACKNOWLEDGEMENTS

First of all I would like to thank the **patients** that have contributed to these studies – without you this thesis would not have been possible! Secondly, I wish to express my sincere gratitude to all of you who have contributed to my work by sharing your expertise, giving your support and last, but not least, by inspiring me. *I especially wish to thank:*

Supervisors/Mentors

Anders Sönnerborg, my supervisor, for sharing your vast knowledge and extraordinary expertise in HIV and science "all around the clock". Thank you also for challenging me out of my "comfort zone", making me grow not only as a researcher, but also in life. ***Veronica Svedhem***, my co-supervisor and colleague in the HIV Clinic, for good collaboration, many nice laughs and for always believing in me, convincing me that what I do is "good enough". ***Gaetano Marrone***, my co-supervisor and statistician, for guidance in the "STATA jungle" and for sharing my enthusiasm for research, life in general and, not to forget, my "Mediterranean temper".

Johan Giesecke, last author in paper I, for introducing me into the joys of epidemiology, public health and science. Your energy and enthusiasm was contagious! ***Karl Ekdahl*** for nice lunch breaks at SMI/EPI in 2003 and for being my external mentor during this thesis.

Co-authors/Collaborators

Anders Blaxhult, for being a very dear friend and an excellent collaborator and co-writer of three of the manuscripts in this thesis. ***Börje Åkerlund***, for pleasant collaboration and for your encouragement about my studies and life. ***Christina Carlander***, for your friendship and your positive energy – I need no yoga when you are around! ***Aylin Yilmaz, Suzanne Wendahl, Nils Kuylenstierna, Örjan Andersson, Petra Tunbäck, Åsa Mellgren, Mats Ericsson, Per Björkman, Farshad Azimi*** with ***colleagues and coworkers at the Swedish HIV clinics*** for your fruitful and inspirational collaboration. ***Ujjwal Neogi*** for kindly introducing me into the mysteries of molecular epidemiology.

Malin Arneborn, former register holder at SMI and ***Eva-Lena Fredriksson***, research nurse and InfCare expert, at the HIV clinic, for your brilliant help and support with my studies– always with a positive attitude, fast delivering at a high quality, whatever the demand.

Torsten Berglund, Maria Axelsson, Lousie Mannheimer and Charlotte Deogan with coworkers for valuable and appreciated input on my projects and for sharing data from the *Public Health Agency of Sweden.* ***Lars Eriksson*** and ***Maria Wiklander,*** Karolinska Institutet*,* for inspiring co-operation on the barriers to testing.

My present and former PhD student colleagues at the Clinic of Infectious Diseases and ***the Sönnerborg research group***, for encouragement, scientific discussions and occasionally a well deserved dinner. ***Jenny Stenkvist*** for your friendship and for always being there, with your rare combination of a brilliant mind, generous heart and great sense of fairness. ***Jan Vesterbacka*** for your scientific and emotional support. ***Hilmir Asgeirsson, Anna Linnér*** and ***Malin Ackefors***, for your encouragement and guidance in the dissertation process. ***Emmi Andersson*** and ***Amanda Häggblom*** for your kind help in extracting data from the InfCare HIV. ***Martin Kåberg*** for your valuable and "addictive" input on PWID.

In Education

Karin Månsson, my biology teacher in the 80's, for inspiring me to learn more about medicine and human nature and for later on also becoming a close friend. ***Jonas Hedlund*** and ***Tony Carlsson*** for attracting my interest in Infectious Disease medicine, being my teaching assistants at Med school in 1996. ***Robert Schvarzc,*** for your great skill to make the complicated simple and for early convincing me that what is moving in the Swedish forest is not very likely to be a zebra, even though I'd still like to see one! ***Olof Akre, Anders Ekbom*** **(**KI and the Research school for clinicians in epidemiology 2004-06) for adding value and new aspects to the life in the clinic.

Bo Hejdeman, Torsten Berglund *and* ***Anna-Mia Ekström*****,** for valuable input and encouragement half time through my PhD-process.

In the clinic

Lena Dillner, Ywonne Lindquist, Lennart Östlund and Anette Pedersen for allowing time for research. A special thanks to you ***Ywonne***, for your generous support and understanding when my puzzle of research, clinical work, family life and own ailments just did not want to make the ends meet.

All present and previous colleagues in the HIV clinic for your inspirational cooperation and excellent collaboration over the years and for your help with the inclusion of patients in these studies. ***P-O Persson***, for many nice rides on the bus from Västerbroplan to Huddinge, 2000-2003, and for early encouraging my interest in HIV. ***Stefan Lindbäck***, for teaching me the basics in HIV medicine and letting me inherit your group of very well cared for patients. ***Anders Thalme*** for your clinical skills with an ever-open mind, practical sense and present heart, being my role model in the clinic.

All ***the staff at the Karolinska HIV Unit, I56*****,** for your contribution to my work, continuous enthusiasm and support, many good laughs and for taking a very good care of our patients. You make the staff room at I56 feel like a second living room, coming home!

Agneta Samuelsson*,* ***Ann Quist****,* ***Illona Llewensohn-Fuchs*** *and* ***Zhibing Yun*** for your professional skills and positive attitude taking care of our analysis in the lab of Microbiology.

All wonderful colleagues, personnel and friends at the Clinics of Infectious diseases in Stockholm*,* in particular my ***doc companions in Huddinge*** for your kindness and support in covering up my absence in daily clinical work – You all are fantastic! ***Karin Hansson*****,** for being one of my dearest friends since the first Friday Pub at "Medicinska Föreningen" in 1992. ***Maria Norrby*** for being the best of room-mates, sharing and enduring my moments of happiness as well as the more gloomy ones for so many years. ***Kerstin Karkkonen*** and ***Elda Sparrelid*** for friendship & clinical mentorship. ***Gudmundur Axelsson*** and ***Lars Mattsson****,* for years of very wise and inspirational clinical guidance. ***Kiki Rönnbäck, Anna Westman, Cina Missalidis*** for your exuberant and contagious energy **–** I miss you! Joint after-work is a brilliant idea!

Friends & family

For being there, for being who you are and for letting me be who I am.

My many dear friends (*no one mentioned, no one forgotten*) that I still hope will be there, after my last year of omission - You all are amazing! It is payback time.

My great, Grand Family - You mean everything to me! Special thanks to my mother-in-law ***Agneta*** for your outstanding support with Aline & Olivia this last year – It has been invaluable!

My grandmother Britt-Mari for being just YOU, always making me feel at ease, no matter what is going on. ***My late grand father Sune***, for endless vivid discussions about just about anything and for sharing my interest in "intellectual puzzles". I wish you were still here.

My sister Emma and ***my "big-sister", auntie Maria****,* for always being there when I need you the most; ***Emma*** for your great sense of humor and for being the most genuine and sincere person I know; ***Maria*** for bringing me magic – I still think I will find the pirate's treasure…

My parents, for LIFE and for the endless love and support you offer my family and me; ***My mother Gunilla*** for your empathic and open heart, being the best listener at all times. ***My father Bosse***, for sharing your positive attitude to life, where nothing is impossible, and for your curiosity; inspiring me to always look behind the next corner.

Christel, my fabulous wife! For better or worse, for richer or poorer, in sickness and in health… and through a whole PhD process. Thank you for sharing my life and for making me smile. I love You!

Aline & Olivia – The miracles of My life, for reminding me of what it is really all about!

This work was supported by grants from the Swedish National Board of Health and Welfare, Public Health Agency of Sweden, Karolinska Institutet, Swedish Physicians against AIDS Foundation, Swedish Society of Infectious Diseases and Gilead Science Nordic.

11 REFERENCES

1. Mocroft, A., et al., *Risk Factors and Outcomes for Late Presentation for HIV-Positive Persons in Europe: Results from the Collaboration of Observational HIV Epidemiological Research Europe Study (COHERE).* PLoS Med, 2013. **10**(9): p. e1001510.
2. Antinori, A., et al., *Late presentation of HIV infection: a consensus definition.* HIV Med, 2011. **12**(1): p. 61-4.
3. Moreno, S., A. Mocroft, and A. Monforte, *Medical and societal consequences of late presentation.* Antivir Ther, 2010. **15 Suppl 1**: p. 9-15.
4. Marks, G., N. Crepaz, and R.S. Janssen, *Estimating sexual transmission of HIV from persons aware and unaware that they are infected with the virus in the USA.* AIDS, 2006. **20**(10): p. 1447-50.
5. Krentz, H.B. and M.J. Gill, *The Direct Medical Costs of Late Presentation (<350/mm) of HIV Infection over a 15-Year Period.* AIDS Res Treat, 2012. **2012**: p. 757135.
6. Barre-Sinoussi, F., A.L. Ross, and J.F. Delfraissy, *Past, present and future: 30 years of HIV research.* Nat Rev Microbiol, 2013. **11**(12): p. 877-83.
7. Centers for Disease Control, *Pneumocystis pneumonia--Los Angeles.* MMWR Morb Mortal Wkly Rep, 1981. **30**(21): p. 250-2.
8. Centers for Disease Control, *Kaposi's sarcoma and Pneumocystis pneumonia among homosexual men-New York City and California.* MMWR Morb Mortal Wkly Rep. , 1981. **30**: p. 305-308.
9. *Epidemiologic aspects of the current outbreak of Kaposi's sarcoma and opportunistic infections.* N Engl J Med, 1982. **306**(4): p. 248-52.
10. Siegal, F.P., et al., *Severe acquired immunodeficiency in male homosexuals, manifested by chronic perianal ulcerative herpes simplex lesions.* N Engl J Med, 1981. **305**(24): p. 1439-44.
11. Gottlieb, M.S., et al., *Pneumocystis carinii pneumonia and mucosal candidiasis in previously healthy homosexual men: evidence of a new acquired cellular immunodeficiency.* N Engl J Med, 1981. **305**(24): p. 1425-31.
12. Greene, W.C., *A history of AIDS: looking back to see ahead.* Eur J Immunol, 2007. **37 Suppl 1**: p. S94-102.
13. Serwadda, D., et al., *Slim disease: a new disease in Uganda and its association with HTLV-III infection.* Lancet, 1985. **2**(8460): p. 849-52.
14. Sharp, P.M. and B.H. Hahn, *The evolution of HIV-1 and the origin of AIDS.* Philos Trans R Soc Lond B Biol Sci, 2010. **365**(1552): p. 2487-94.
15. Gallo, R.C., et al., *Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS.* Science, 1984. **224**(4648): p. 500-3.
16. Barre-Sinoussi, F., et al., *Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS).* Science, 1983. **220**(4599): p. 868-71.
17. Safai, B., et al., *Seroepidemiological studies of human T-lymphotropic retrovirus type III in acquired immunodeficiency syndrome.* Lancet, 1984. **1**(8392): p. 1438-40.
18. Sarngadharan, M.G., et al., *Antibodies reactive with human T-lymphotropic retroviruses (HTLV-III) in the serum of patients with AIDS.* Science, 1984. **224**(4648): p. 506-8.
19. Marwick, C., *AZT (zidovudine) just a step away from FDA approval for AIDS therapy.* JAMA, 1987. **257**(10): p. 1281-2.

20. Baker, R., *FDA approves 3TC and saquinavir. Food and Drug Administration.* BETA, 1995: p. 5, 9.
21. Mellors, J.W., et al., *Prognosis in HIV-1 infection predicted by the quantity of virus in plasma.* Science, 1996. **272**(5265): p. 1167-70.
22. Chiu, I.M., et al., *Nucleotide sequence evidence for relationship of AIDS retrovirus to lentiviruses.* Nature, 1985. **317**(6035): p. 366-8.
23. Sundquist, W.I. and H.G. Krausslich, *HIV-1 assembly, budding, and maturation.* Cold Spring Harb Perspect Med, 2012. **2**(7): p. a006924.
24. Frankel, A.D. and J.A. Young, *HIV-1: fifteen proteins and an RNA.* Annu Rev Biochem, 1998. **67**: p. 1-25.
25. Robertson, D.L., et al., *HIV-1 nomenclature proposal.* Science, 2000. **288**(5463): p. 55-6.
26. Ayehunie, S., et al., *New subtype of HIV-1 in Ethiopia.* Lancet, 1990. **336**(8720): p. 942.
27. Hemelaar, J., *The origin and diversity of the HIV-1 pandemic.* Trends Mol Med, 2012. **18**(3): p. 182-92.
28. Neogi, U., et al., *Temporal trends in the Swedish HIV-1 epidemic: increase in non-B subtypes and recombinant forms over three decades.* PLoS One, 2014. **9**(6): p. e99390.
29. Moir, S., T.W. Chun, and A.S. Fauci, *Pathogenic mechanisms of HIV disease.* Annu Rev Pathol, 2011. **6**: p. 223-48.
30. Masur, H., et al., *CD4 counts as predictors of opportunistic pneumonias in human immunodeficiency virus (HIV) infection.* Ann Intern Med, 1989. **111**(3): p. 223-31.
31. Buggert, M., et al., *Multiparametric bioinformatics distinguish the CD4/CD8 ratio as a suitable laboratory predictor of combined T cell pathogenesis in HIV infection.* J Immunol, 2014. **192**(5): p. 2099-108.
32. Msellati, P., et al., *A cohort study of 89 HIV-1-infected adult patients contaminated by blood products: Bordeaux 1981-1989. Groupe d'Epidemiologie Clinique du SIDA en Aquitaine (GECSA).* AIDS, 1990. **4**(11): p. 1105-9.
33. Public Health Agency of Canada, *HIV Transmission Risk: A Summary of Evidence. Available at:* http://www.phac-aspc.gc.ca/aids-sida/publication/hivtr-rtvih-eng.php. *Accessed 29/1/2016.* 2013.
34. Centre for Disese Control and prevention. *HIV Transmission Risk. Available at:* http://www.cdc.gov/hiv/policies/law/risk.html. *Accessed 20/1/2016.* 2015.
35. Albert, J., et al., *Risk of HIV transmission from patients on antiretroviral therapy: a position statement from the Public Health Agency of Sweden and the Swedish Reference Group for Antiviral Therapy.* Scand J Infect Dis, 2014. **46**(10): p. 673-7.
36. Borrow, P., et al., *Innate immunity against HIV: a priority target for HIV prevention research.* Retrovirology, 2010. **7**: p. 84.
37. Siliciano, R.F. and W.C. Greene, *HIV latency.* Cold Spring Harb Perspect Med, 2011. **1**(1): p. a007096.
38. Lindback, S., et al., *Viral dynamics in primary HIV-1 infection. Karolinska Institutet Primary HIV Infection Study Group.* AIDS, 2000. **14**(15): p. 2283-91.
39. Lackner, A.A., M.M. Lederman, and B. Rodriguez, *HIV pathogenesis: the host.* Cold Spring Harb Perspect Med, 2012. **2**(9): p. a007005.
40. Pilcher, C.D., et al., *Acute HIV revisited: new opportunities for treatment and prevention.* J Clin Invest, 2004. **113**(7): p. 937-45.
41. Doitsh, G., et al., *Cell death by pyroptosis drives CD4 T-cell depletion in HIV-1 infection.* Nature, 2014. **505**(7484): p. 509-14.
42. Buchbinder, S. and E. Vittinghoff, *HIV-infected long-term nonprogressors: epidemiology, mechanisms of delayed progression, and clinical and research implications.* Microbes Infect, 1999. **1**(13): p. 1113-20.

43. Pantaleo, G., C. Graziosi, and A.S. Fauci, *New concepts in the immunopathogenesis of human immunodeficiency virus infection.* N Engl J Med, 1993. **328**(5): p. 327-35.
44. Gail, M.H., P.S. Rosenberg, and J.J. Goedert, *Therapy may explain recent deficits in AIDS incidence.* J Acquir Immune Defic Syndr, 1990. **3**(4): p. 296-306.
45. CDC, *Centers for Disease Control and Prevention. 1993 revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults.* MMWR Recomm Rep, 1992. **41**(RR-17): p. 1-19.
46. Collaboration of Observational H. I. V. Epidemiological Research Europe in EuroCoord, et al., *All-cause mortality in treated HIV-infected adults with CD4 >/=500/mm3 compared with the general population: evidence from a large European observational cohort collaboration.* Int J Epidemiol, 2012. **41**(2): p. 433-45.
47. Gaines, H., et al., *Six-week follow-up after HIV-1 exposure: a position statement from the Public Health Agency of Sweden and the Swedish Reference Group for Antiviral Therapy.* Infect Dis (Lond), 2016. **48**(2): p. 93-8.
48. Eyawo, O., R.S. Hogg, and J.S. Montaner, *The Holy Grail: The search for undiagnosed cases is paramount in improving the cascade of care among people living with HIV.* Can J Public Health, 2013. **104**(5): p. e418-9.
49. Hiv-Causal Collaboration, et al., *The effect of combined antiretroviral therapy on the overall mortality of HIV-infected individuals.* AIDS, 2010. **24**(1): p. 123-37.
50. Nakagawa, F., et al., *Projected life expectancy of people with HIV according to timing of diagnosis.* AIDS, 2012. **26**(3): p. 335-43.
51. Antiretroviral Therapy Cohort, C., *Life expectancy of individuals on combination antiretroviral therapy in high-income countries: a collaborative analysis of 14 cohort studies.* Lancet, 2008. **372**(9635): p. 293-9.
52. Lohse, N., et al., *Survival of persons with and without HIV infection in Denmark, 1995-2005.* Ann Intern Med, 2007. **146**(2): p. 87-95.
53. van Sighem, A.I., et al., *Life expectancy of recently diagnosed asymptomatic HIV-infected patients approaches that of uninfected individuals.* AIDS, 2010. **24**(10): p. 1527-35.
54. Cahn, P., et al., *Dual therapy with lopinavir and ritonavir plus lamivudine versus triple therapy with lopinavir and ritonavir plus two nucleoside reverse transcriptase inhibitors in antiretroviral-therapy-naive adults with HIV-1 infection: 48 week results of the randomised, open label, non-inferiority GARDEL trial.* Lancet Infect Dis, 2014. **14**(7): p. 572-80.
55. Gatell., J.M., et al., *Non-inferiority of dual-therapy (DT) with lopinavir/ritonavir (LPV/r) plus lamivudine (3TC) vs. triple-therapy (TT) with LPV/r plus two nucleos(t)ides (NRTIs) for maintenance of HIV viral suppression: 48-week results of OLE study. Abstract LBPE17 IAC 2014.*
56. Perez-Molina, J.A., et al., *Switching to dual therapy (atazanavir/ritonavir+lamivudine) vs. standard triple therapy (atazanavir/ritonavir+2 nucleos[t]ides) is safe and effective in virologically suppressed patients: 48-week results of a randomized clinical trial (SALT study). Abstract LBPE18 IAC 2014.*
57. Figueroa MI, et al., *Dolutegravir-lamivudine as initial therapy in HIV-infected ARV-naive patients: first results of the PADDLE trial. Program and abstracts of the 15th European AIDS Conference; October 21-24, 2015; Barcelona, Spain. Abstract 1066.* 2015.
58. Insight Start Study Group, et al., *Initiation of Antiretroviral Therapy in Early Asymptomatic HIV Infection.* N Engl J Med, 2015. **373**(9): p. 795-807.
59. Temprano Anrs Study Group, et al., *A Trial of Early Antiretrovirals and Isoniazid Preventive Therapy in Africa.* N Engl J Med, 2015. **373**(9): p. 808-22.

60. WHO. *World Health Organization. Guideline on when to start antiretroviral therapy and on pre-exposure prophylaxis for HIV 2015. ISBN: 978 92 4 150956 5. Available at:* http://www.who.int/hiv/pub/guidelines/earlyrelease-arv/en/. *Accessed 15/11/2015.*
61. European AIDS Clinical Society. *EACS Guidelines 8.0. Available at:* http://www.eacsociety.org/files/2015_eacsguidelines_8.0-english_rev-20151221.pdf. *Accessed 05/01/16*. 2015.
62. Panel on Antiretroviral Guidelines for Adults and Adolescents. *Guidelines for the use of an- tiretroviral agents in HIV-1-infected adults and adolescents. Department of Health and Human Services. Available at:* http://www.aidsinfo.nih.gov/ContentFiles/AdultandAdoles- *centGL.pdf. Accessed 5/1/2016.* 2015.
63. Tenorio, A.R., et al., *Soluble markers of inflammation and coagulation but not T-cell activation predict non-AIDS-defining morbid events during suppressive antiretroviral treatment.* J Infect Dis, 2014. **210**(8): p. 1248-59.
64. McComsey, G.A., et al., *Associations of inflammatory markers with AIDS and non-AIDS clinical events after initiation of antiretroviral therapy: AIDS clinical trials group A5224s, a substudy of ACTG A5202.* J Acquir Immune Defic Syndr, 2014. **65**(2): p. 167-74.
65. French, M.A., et al., *Serum immune activation markers are persistently increased in patients with HIV infection after 6 years of antiretroviral therapy despite suppression of viral replication and reconstitution of CD4+ T cells.* J Infect Dis, 2009. **200**(8): p. 1212-5.
66. Hunt, P.W., et al., *T cell activation is associated with lower CD4+ T cell gains in human immunodeficiency virus-infected patients with sustained viral suppression during antiretroviral therapy.* J Infect Dis, 2003. **187**(10): p. 1534-43.
67. Hutter, G., et al., *Long-term control of HIV by CCR5 Delta32/Delta32 stem-cell transplantation.* N Engl J Med, 2009. **360**(7): p. 692-8.
68. Rasmussen, T.A., M. Tolstrup, and O.S. Sogaard, *Reversal of Latency as Part of a Cure for HIV-1.* Trends Microbiol, 2015.
69. Siliciano, J.D. and R.F. Siliciano, *Recent developments in the search for a cure for HIV-1 infection: targeting the latent reservoir for HIV-1.* J Allergy Clin Immunol, 2014. **134**(1): p. 12-9.
70. Giordano, T.P., M.E. Suarez-Almazor, and R.M. Grimes, *The population effectiveness of highly active antiretroviral therapy: are good drugs good enough?* Curr HIV/AIDS Rep, 2005. **2**(4): p. 177-83.
71. Ulett, K.B., et al., *The therapeutic implications of timely linkage and early retention in HIV care.* AIDS Patient Care STDS, 2009. **23**(1): p. 41-9.
72. Gardner, E.M., et al., *The spectrum of engagement in HIV care and its relevance to test-and-treat strategies for prevention of HIV infection.* Clin Infect Dis, 2011. **52**(6): p. 793-800.
73. UNAIDS, *90–90–90 - An ambitious treatment target to help end the AIDS epidemic. Available at:* http://www.unaids.org/en/resources/documents/2014/90-90-90. *Accessed 19/11/2015.* 2014.
74. UNAIDS, *UNAIDS factsheet: Global statistics 2014. Available at* http://www.unaids.org/en/resources/campaigns/HowAIDSchangedeverything/factsheet. *Accessed 11/11/2015*. 2015.
75. UNAIDS, *UNAIDS Global Report 2013. Available at:* http://www.unaids.org/sites/default/files/media_asset/UNAIDS_Global_Report_2013_en_1.pdf. *Accessed 6/11/2015.* 2013.
76. World Health Organization. *WHO: HIV/AIDS. Available at* http://www.who.int/hiv/en/. *Accessed 11/11/2015.*

77. Abu-Raddad, L.J., et al., *Epidemiology of HIV infection in the Middle East and North Africa.* AIDS, 2010. **24 Suppl 2**: p. S5-23.
78. United Nations, *Resolution adopted by the General Assembly; 55/2. United Nations Millennium Declaration.* http://www.un.org/millennium/declaration/ares552e.htm. *Accesed 28/12/2015.* 2000.
79. The Global Fund, *The Global Fund annual report 2002 - 2003. Available at:* http://www.theglobalfund.org/en/archive/annualreports/. *Accessed 28/12/2015.* 2003.
80. US Department of State, *PEPFAR blueprint: creating an AIDS-free Generation. Available at:* http://www.pepfar.gov/documents/organization/201386.pdf. *Accessed 28/12/2015.*
81. UNAIDS, *How AIDS changed everything — MDG6: 15 years, 15 lessons of hope from the AIDS response. Available at:* http://www.unaids.org/en/resources/documents/2015/MDG6_15years-15lessonsfromtheAIDSresponse. *Accessed 18/11/2015.* 2015.
82. WHO., *World Health Organization. Guideline on when to start antiretroviral therapy and on pre-exposure prophylaxis for HIV. Available at:* http://www.who.int/hiv/pub/guidelines/earlyrelease-arv/en/. *Accessed 20/11/2015.* 2015.
83. UNAIDS. *UNAIDS Gap Report. Available at:* http://www.unaids.org/sites/default/files/en/media/unaids/contentassets/documents/unaidspublication/2014/UNAIDS_Gap_report_en.pdf. *Accessed 28/12/2015.* 2014.
84. European Centre for Disease Prevention and Control (ECDC), *From Dublin to Rome: ten years of responding to HIV in Europe and Central Asia. Available at:* http://www.ecdc.europa.eu/en/publications/Publications/dublin-rome-10-years-hiv-europe-central-asia.pdf . *Accessed 18/11/2015.* 2014.
85. Pharris, A., et al., *Ten years after Dublin: principal trends in HIV surveillance in the EU/EEA, 2004 to 2013.* Euro Surveill, 2014. **19**(47): p. 20968.
86. ECDC and WHO Regional Office for Europe, *HIV/AIDS Surveillance in Europe 2014. Available at:* http://ecdc.europa.eu/en/publications/Publications/hiv-aids-surveillance-in-Europe-2014.pdf. *Accessed 27/1/2016.* 2015.
87. Nakagawa, F., A.N. Phillips, and J.D. Lundgren, *Update on HIV in Western Europe.* Curr HIV/AIDS Rep, 2014. **11**(2): p. 177-85.
88. Hernando, V., et al., *HIV Infection in Migrant Populations in the European Union and European Economic Area in 2007-2012: An Epidemic on the Move.* J Acquir Immune Defic Syndr, 2015. **70**(2): p. 204-11.
89. European Centre for Disease Prevention and Control, *Migrant health: Sexual transmission of HIV within migrant groups in the EU/EEA and implications for effective interventions. Available at:* http://ecdc.europa.eu/en/publications/Publications/Migrant-health-sexual-transmission.pdf. *Accessed 4/11/2015.* 2013.
90. Fakoya, I., et al., *A systematic review of post-migration acquisition of HIV among migrants from countries with generalised HIV epidemics living in Europe: Implications for effectively managing HIV prevention programmes and policy.* BMC Public Health, 2015. **15**: p. 561.
91. UNAIDS, *UNAIDS Factsheet Global Statistics 2015. Available at:* http://www.unaids.org/sites/default/files/media_asset/20150901_FactSheet_2015_en.pdf. *Accessed 28/12/2015.* 2015.
92. Pehrson, P., et al., *AIDS bland homosexuella män: De första svenska fallen.* Läkartidningen 80:545-8., 1983.
93. Berglund, T., M. Arneborn, and J. Giesecke, *Bakgrundsdokumentation Information från läkemedelsverket 5:2002. Available at https://lakemedelsverket.se/upload/om-*

lakemedelsverket/publikationer/information-fran-lakemedelsverket/Info_fr_LV_2002-5.pdf. Accessed 14/1/2016. 2002.
94. Sveriges Riksdag, *Svensk författningssamling 2004:168; Smittskyddslag (2004:168). Available at* http://www.riksdagen.se/sv/Dokument-Lagar/Lagar/Svenskforfattningssamling/Smittskyddslag-2004168_sfs-2004-168/. *Accessed 11/11/2015.* 2004.
95. Statistics Sweden (SCB), *Population statistics 2014. Available at:* http://www.scb.se/en_/Finding-statistics/Statistics-by-subject-area/Population. *Accessed 18/1/2016.*
96. Rice, B.D., et al., *A new method to assign country of HIV infection among heterosexuals born abroad and diagnosed with HIV.* AIDS, 2012. **26**(15): p. 1961-6.
97. Referensgruppen för Antiretroviral Terapi (RAV). *Antiretroviral behandling av HIV-infektion 2014 – Behandlingsrekommendation. Available from* http://www.RAV.nu. *Accessed 11/01/2016*. 2014.
98. Gisslén M, et al., *The UNAIDS/WHO 90-90-90 targets for HIV care and treatment cascade reached in Sweden. Manuscript in Preparation 2016.*
99. European Centre for Disease Prevention and Control, *ECDC Special Report; Thematic report: HIV continuum of care. Monitoring implementation of the Dublin Declaration on Partnership to Fight HIV/AIDS in Europe and Central Asia: 2014 progress report. Available at:* http://ecdc.europa.eu/en/publications/Publications/dublin-declaration-continuum-of-care-2014.pdf. *Accessed 28/12/2015.* 2014.
100. Adler, A., S. Mounier-Jack, and R.J. Coker, *Late diagnosis of HIV in Europe: definitional and public health challenges.* AIDS Care, 2009. **21**(3): p. 284-93.
101. Antinori, A., et al., *Report of a European Working Group on late presentation with HIV infection: recommendations and regional variation.* Antivir Ther, 2010. **15 Suppl 1**: p. 31-5.
102. Delpierre, C., et al., *Characteristics trends, mortality and morbidity in persons newly diagnosed HIV positive during the last decade: the profile of new HIV diagnosed people.* Eur J Public Health, 2008. **18**(3): p. 345-7.
103. Fisher, M., *Late diagnosis of HIV infection: major consequences and missed opportunities.* Curr Opin Infect Dis, 2008. **21**(1): p. 1-3.
104. Girardi, E., C.A. Sabin, and A.D. Monforte, *Late diagnosis of HIV infection: epidemiological features, consequences and strategies to encourage earlier testing.* J Acquir Immune Defic Syndr, 2007. **46 Suppl 1**: p. S3-8.
105. Sabin, C.A., et al., *Late diagnosis in the HAART era: proposed common definitions and associations with mortality.* AIDS, 2010. **24**(5): p. 723-7.
106. Waters, L. and C.A. Sabin, *Late HIV presentation: epidemiology, clinical implications and management.* Expert Rev Anti Infect Ther, 2011. **9**(10): p. 877-89.
107. May, M., et al., *Impact of late diagnosis and treatment on life expectancy in people with HIV-1: UK Collaborative HIV Cohort (UK CHIC) Study.* BMJ, 2011. **343**: p. d6016.
108. Bhaskaran, K., et al., *Changes in the risk of death after HIV seroconversion compared with mortality in the general population.* JAMA, 2008. **300**(1): p. 51-9.
109. Collaboration;, A.T.C., *Life expectancy of individuals on combination antiretroviral therapy in high-income countries: a collaborative analysis of 14 cohort studies.* Lancet, 2008. **372**(9635): p. 293-9.
110. Castilla, J., et al., *Late diagnosis of HIV infection in the era of highly active antiretroviral therapy: consequences for AIDS incidence.* AIDS, 2002. **16**(14): p. 1945-51.
111. Girardi, E., et al., *Increasing proportion of late diagnosis of HIV infection among patients with AIDS in Italy following introduction of combination antiretroviral therapy.* J Acquir Immune Defic Syndr, 2000. **25**(1): p. 71-6.

112. Hocking, J.S., et al., *Late presentation of HIV infection associated with prolonged survival following AIDS diagnosis--characteristics of individuals.* Int J STD AIDS, 2000. **11**(8): p. 503-8.
113. Centers for Disease Control Prevention, *Late versus early testing of HIV--16 Sites, United States, 2000-2003.* MMWR Morb Mortal Wkly Rep, 2003. **52**(25): p. 581-6.
114. Branson, B.M., et al., *Revised recommendations for HIV testing of adults, adolescents, and pregnant women in health-care settings.* MMWR Recomm Rep, 2006. **55**(RR-14): p. 1-17; quiz CE1-4.
115. Nakao, J.H., et al., *Falling through the cracks? Missed opportunities for earlier HIV diagnosis in a New York City Hospital.* Int J STD AIDS, 2014. **25**(12): p. 887-93.
116. d'Almeida, K.W., et al., *Modest public health impact of nontargeted human immunodeficiency virus screening in 29 emergency departments.* Arch Intern Med, 2012. **172**(1): p. 12-20.
117. Coenen, T., et al., *Optimal HIV testing and earlier care: the way forward in Europe.* HIV Med, 2008. **9 Suppl 2**: p. 1-5.
118. Egnell, K. and V. Svedhem Johansson, *23% of newly diagnosed HIV cases 2007 in Karolinska University hospital had opportunistic infections. P 256*, in *The Ninth International Congress on Drug Therapy in HIV Infection, Glasgow 2009*. 2009.
119. Clumeck, N., et al., *European AIDS Clinical Society (EACS) guidelines for the clinical management and treatment of HIV-infected adults.* HIV Med, 2008. **9**(2): p. 65-71.
120. Hamers, F.F. and A.N. Phillips, *Diagnosed and undiagnosed HIV-infected populations in Europe.* HIV Med, 2008. **9 Suppl 2**: p. 6-12.
121. Raymond, A., A. Hill, and A. Pozniak, *Large disparities in HIV treatment cascades between eight European and high-income countries – analysis of break points. Abstracts of the HIV Drug Therapy Glasgow Congress 2014. .* Journal of the International AIDS Society 2014, 17(Suppl 3):19507., 2014.
122. Cheever, L.W., *Engaging HIV-infected patients in care: their lives depend on it.* Clin Infect Dis, 2007. **44**(11): p. 1500-2.
123. *EPI-aktuellt, vol 6, nr 42 (18 oktober 2007). Available at* http://www.folkhalsomyndigheten.se*. Accessed 151020.*
124. Working Group on Estimation of, H.I.V.P.i.E., *HIV in hiding: methods and data requirements for the estimation of the number of people living with undiagnosed HIV.* AIDS, 2011. **25**(8): p. 1017-23.
125. Hall, H.I., et al., *Estimation of HIV incidence in the United States.* JAMA, 2008. **300**(5): p. 520-9.
126. Sommen, C., A. Alioum, and D. Commenges, *A multistate approach for estimating the incidence of human immunodeficiency virus by using HIV and AIDS French surveillance data.* Stat Med, 2009. **28**(11): p. 1554-68.
127. Sweeting, M.J., D. De Angelis, and O.O. Aalen, *Bayesian back-calculation using a multi-state model with application to HIV.* Stat Med, 2005. **24**(24): p. 3991-4007.
128. Wand, H., et al., *Increasing HIV transmission through male homosexual and heterosexual contact in Australia: results from an extended back-projection approach.* HIV Med, 2010. **11**(6): p. 395-403.
129. van Sighem, A., et al., *Estimating HIV Incidence, Time to Diagnosis, and the Undiagnosed HIV Epidemic Using Routine Surveillance Data.* Epidemiology, 2015. **26**(5): p. 653-60.
130. Lodwick, R.K., et al., *Use of surveillance data on HIV diagnoses with HIV-related symptoms to estimate the number of people living with undiagnosed HIV in need of antiretroviral therapy.* PLoS One, 2015. **10**(3): p. e0121992.

131. Barnighausen, T., et al., *HIV incidence estimation using the BED capture enzyme immunoassay: systematic review and sensitivity analysis.* Epidemiology, 2010. **21**(5): p. 685-97.
132. Widgren, K., et al., *Delayed HIV diagnosis common in Sweden, 2003-2010.* Scand J Infect Dis, 2014: p. 1-6.
133. ECDC, *European Centre for Disease Prevention and Control. Technical Report. Monitoring recently acquired HIV infections in the European context. Stockholm: ECDC; 2013. Available at:* http://ecdc.europa.eu/en/publications/Publications/monitoring-recently-acquired-HIV-infections-european-context.pdf *Accessed 14/1/2016.*
134. Moore, R.D., *Epidemiology of HIV infection in the United States: implications for linkage to care.* Clin Infect Dis, 2011. **52 Suppl 2**: p. S208-13.
135. Lucas, S.B., H. Curtis, and M.A. Johnson, *National review of deaths among HIV-infected adults.* Clin Med, 2008. **8**(3): p. 250-2.
136. Egger, M., et al., *Prognosis of HIV-1-infected patients starting highly active antiretroviral therapy: a collaborative analysis of prospective studies.* Lancet, 2002. **360**(9327): p. 119-29.
137. Brown, A.E., et al., *Auditing national HIV guidelines and policies: The United Kingdom CD4 Surveillance Scheme.* Open AIDS J, 2012. **6**: p. 149-55.
138. Deeks, S.G., et al., *Immune activation set point during early HIV infection predicts subsequent CD4+ T-cell changes independent of viral load.* Blood, 2004. **104**(4): p. 942-7.
139. Kelley, C.F., et al., *Incomplete peripheral CD4+ cell count restoration in HIV-infected patients receiving long-term antiretroviral treatment.* Clin Infect Dis, 2009. **48**(6): p. 787-94.
140. Guaraldi, G., et al., *Premature age-related comorbidities among HIV-infected persons compared with the general population.* Clin Infect Dis, 2011. **53**(11): p. 1120-6.
141. Archin, N.M., et al., *Immediate antiviral therapy appears to restrict resting CD4+ cell HIV-1 infection without accelerating the decay of latent infection.* Proc Natl Acad Sci U S A, 2012. **109**(24): p. 9523-8.
142. Rockstroh, J.K., et al., *Management of late-presenting patients with HIV infection.* Antivir Ther, 2010. **15 Suppl 1**: p. 25-30.
143. Strategies for Management of Antiretroviral Therapy Study Group, et al., *CD4+ count-guided interruption of antiretroviral treatment.* N Engl J Med, 2006. **355**(22): p. 2283-96.
144. Opravil, M., et al., *Clinical efficacy of early initiation of HAART in patients with asymptomatic HIV infection and CD4 cell count > 350 x 10(6) /l.* AIDS, 2002. **16**(10): p. 1371-81.
145. When To Start Consortium, et al., *Timing of initiation of antiretroviral therapy in AIDS-free HIV-1-infected patients: a collaborative analysis of 18 HIV cohort studies.* Lancet, 2009. **373**(9672): p. 1352-63.
146. Wawer, M.J., et al., *Rates of HIV-1 transmission per coital act, by stage of HIV-1 infection, in Rakai, Uganda.* J Infect Dis, 2005. **191**(9): p. 1403-9.
147. Brenner, B.G., et al., *High rates of forward transmission events after acute/early HIV-1 infection.* J Infect Dis, 2007. **195**(7): p. 951-9.
148. Marks, G., et al., *Meta-analysis of high-risk sexual behavior in persons aware and unaware they are infected with HIV in the United States: implications for HIV prevention programs.* J Acquir Immune Defic Syndr, 2005. **39**(4): p. 446-53.
149. Weinhardt, L.S., et al., *Effects of HIV counseling and testing on sexual risk behavior: a meta-analytic review of published research, 1985-1997.* Am J Public Health, 1999. **89**(9): p. 1397-405.

150. Crepaz, N., et al., *Prevalence of unprotected anal intercourse among HIV-diagnosed MSM in the United States: a meta-analysis.* AIDS, 2009. **23**(13): p. 1617-29.
151. Fox, J., et al., *Reductions in HIV transmission risk behaviour following diagnosis of primary HIV infection: a cohort of high-risk men who have sex with men.* HIV Med, 2009. **10**(7): p. 432-8.
152. Vernazza P et al, *Les personnes séropositives ne souffrant d'aucune autre MST et suivant un traitment antirétroviral efficace ne transmettent pas le VIH par voie sexuelle.* Bulletin des médecins suisses 89 (5). English translation, including translator's affidavit, available at: http://tinyurl.com/cpyt5n, 2008. **89**(5).
153. Castilla, J., et al., *Effectiveness of highly active antiretroviral therapy in reducing heterosexual transmission of HIV*. J Acquir Immune Defic Syndr, 2005. **40**(1): p. 96-101.
154. Mandelbrot, L., et al., *No Perinatal HIV-1 Transmission From Women With Effective Antiretroviral Therapy Starting Before Conception.* Clin Infect Dis, 2015. **61**(11): p. 1715-25.
155. Tanser, F., et al., *High coverage of ART associated with decline in risk of HIV acquisition in rural KwaZulu-Natal, South Africa.* Science, 2013. **339**(6122): p. 966-71.
156. Cohen, M.S., et al., *Prevention of HIV-1 infection with early antiretroviral therapy.* N Engl J Med, 2011. **365**(6): p. 493-505.
157. Rodger, A. *HIV transmission risk through condomless sex if HIV+ partner on suppressive ART: PARTNER Study. 21st CROI, 3-6 March 2014, Boston. Oral late breaker abstract 153LB. Available at:* http://www.croiwebcasts.org/console/player/22072*. Accessed 16/11/2015.*
158. Granich, R.M., et al., *Universal voluntary HIV testing with immediate antiretroviral therapy as a strategy for elimination of HIV transmission: a mathematical model.* Lancet, 2009. **373**(9657): p. 48-57.
159. Krentz, H.B. and J. Gill, *Despite CD4 cell count rebound the higher initial costs of medical care for HIV-infected patients persist 5 years after presentation with CD4 cell counts less than 350 mul.* AIDS, 2010. **24**(17): p. 2750-3.
160. Fleishman, J.A., et al., *The economic burden of late entry into medical care for patients with HIV infection.* Med Care, 2010. **48**(12): p. 1071-9.
161. Brawley, D., et al., *Missed opportunities for HIV diagnosis: a three-year audit in the West of Scotland.* Scott Med J, 2013. **58**(3): p. 173-7.
162. Johnson, M., C. Sabin, and E. Girardi, *Definition and epidemiology of late presentation in Europe.* Antivir Ther, 2010. **15 Suppl 1**: p. 3-8.
163. Girardi, E., et al., *Delayed presentation and late testing for HIV: demographic and behavioral risk factors in a multicenter study in Italy.* J Acquir Immune Defic Syndr, 2004. **36**(4): p. 951-9.
164. Sabin, C.A., et al., *Late presenters in the era of highly active antiretroviral therapy: uptake of and responses to antiretroviral therapy.* AIDS, 2004. **18**(16): p. 2145-51.
165. Lazarus, J.V., et al., *The case for indicator condition-guided HIV screening.* HIV Med, 2013. **14**(7): p. 445-8.
166. Sullivan, A.K., et al., *Feasibility and effectiveness of indicator condition-guided testing for HIV: results from HIDES I (HIV indicator diseases across Europe study).* PLoS One, 2013. **8**(1): p. e52845.
167. Wohlgemut, J., T. Lawes, and R.B. Laing, *Trends in missed presentations and late HIV diagnosis in a UK teaching hospital: a retrospective comparative cohort study.* BMC Infect Dis, 2012. **12**: p. 72.
168. Helleberg, M., et al., *Late presenters, repeated testing, and missed opportunities in a Danish nationwide HIV cohort.* Scand J Infect Dis, 2012. **44**(4): p. 282-8.

169. Liddicoat, R.V., et al., *Assessing missed opportunities for HIV testing in medical settings.* J Gen Intern Med, 2004. **19**(4): p. 349-56.
170. Yazdanpanah, Y., et al., *Routine HIV screening in France: clinical impact and cost-effectiveness.* PLoS One, 2010. **5**(10): p. e13132.
171. Paltiel, A.D., et al., *Expanded screening for HIV in the United States--an analysis of cost-effectiveness.* N Engl J Med, 2005. **352**(6): p. 586-95.
172. Sanders, G.D., et al., *Cost-effectiveness of screening for HIV in the era of highly active antiretroviral therapy.* N Engl J Med, 2005. **352**(6): p. 570-85.
173. Ellis, S., et al., *HIV diagnoses and missed opportunities. Results of the British HIV Association (BHIVA) National Audit 2010.* Clin Med, 2012. **12**(5): p. 430-4.
174. Rayment, M., et al., *HIV testing in non-traditional settings--the HINTS study: a multi-centre observational study of feasibility and acceptability.* PLoS One, 2012. **7**(6): p. e39530.
175. Deblonde, J., et al., *Barriers to HIV testing in Europe: a systematic review.* Eur J Public Health, 2010. **20**(4): p. 422-32.
176. Simmons, E.M., et al., *Barriers and facilitators to HIV testing in primary care among health care providers.* J Natl Med Assoc, 2011. **103**(5): p. 432-8.
177. Burke, R.C., et al., *Why don't physicians test for HIV? A review of the US literature.* AIDS, 2007. **21**(12): p. 1617-24.
178. Korthuis, P.T., et al., *General internists' beliefs, behaviors, and perceived barriers to routine HIV screening in primary care.* AIDS Educ Prev, 2011. **23**(3 Suppl): p. 70-83.
179. Champenois, K., et al., *Missed opportunities for HIV testing in newly-HIV-diagnosed patients, a cross sectional study.* BMC Infect Dis, 2013. **13**: p. 200.
180. de Wit, J.B. and P.C. Adam, *To test or not to test: psychosocial barriers to HIV testing in high-income countries.* HIV Med, 2008. **9 Suppl 2**: p. 20-2.
181. Touloumi, G., et al., *Impact of HIV-1 subtype on CD4 count at HIV seroconversion, rate of decline, and viral load set point in European seroconverter cohorts.* Clin Infect Dis, 2013. **56**(6): p. 888-97.
182. Jarrin, I., et al., *Uptake of combination antiretroviral therapy and HIV disease progression according to geographical origin in seroconverters in Europe, Canada, and Australia.* Clin Infect Dis, 2012. **54**(1): p. 111-8.
183. Volz, E.M. and S.D. Frost, *Inferring the source of transmission with phylogenetic data.* PLoS Comput Biol, 2013. **9**(12): p. e1003397.
184. Ancelle-Park, R., *Expanded European AIDS case definition.* Lancet, 1993. **341**(8842): p. 441.
185. UNAIDS. http://www.unaids.org/en/regionscountries/regions/. 2013; Available from: http://www.unaids.org/en/regionscountries/regions/.
186. World Medical Association, *Declaration of Helsinki. Ethical Principles for Medical Research Involving Human Subjects.* JAMA, 2013. **310**(20): p. 2191-2194.
187. Mocroft, A., et al., *Decline in the AIDS and death rates in the EuroSIDA study: an observational study.* Lancet, 2003. **362**(9377): p. 22-9.
188. Risher, K., K.H. Mayer, and C. Beyrer, *HIV treatment cascade in MSM, people who inject drugs, and sex workers.* Curr Opin HIV AIDS, 2015. **10**(6): p. 420-9.
189. Lesko, C.R., et al., *A systematic review and meta-regression of temporal trends in adult CD4(+) cell count at presentation to HIV care, 1992-2011.* Clin Infect Dis, 2013. **57**(7): p. 1027-37.
190. Late presenters working group in COHERE in EuroCoord, *Late presentation for HIV care across Europe: update from the Collaboration of Observational HIV Epidemiological Research Europe (COHERE) study, 2010 to 2013.* Euro Surveill, 2015. **20**(47).

191. Camoni, L., et al., *Late presenters among persons with a new HIV diagnosis in Italy, 2010--2011.* BMC Public Health, 2013. **13**(1): p. 281.
192. Zoufaly, A., et al., *Late presentation for HIV diagnosis and care in Germany.* HIV Med, 2012. **13**(3): p. 172-81.
193. Vives, N., et al., *Factors associated with late presentation of HIV infection in Catalonia, Spain.* Int J STD AIDS, 2012. **23**(7): p. 475-80.
194. Vincent, A., et al., *Late presentation for HIV diagnosis: a single-centre experience.* J Int AIDS Soc, 2012. **15**(6): p. 18368.
195. d'Arminio Monforte, A., et al., *Late presenters in new HIV diagnoses from an Italian cohort of HIV-infected patients: prevalence and clinical outcome.* Antivir Ther, 2011. **16**(7): p. 1103-12.
196. Ndiaye, B., et al., *Factors associated with presentation to care with advanced HIV disease in Brussels and Northern France: 1997-2007.* BMC Infect Dis, 2011. **11**: p. 11.
197. Tillman, J.L. and H.D. Mark, *HIV and STI testing in older adults: an integrative review.* J Clin Nurs, 2015. **24**(15-16): p. 2074-95.
198. Smith, R.D., et al., *HIV transmission and high rates of late diagnoses among adults aged 50 years and over.* AIDS, 2010. **24**(13): p. 2109-15.
199. Lindau, S.T., et al., *A study of sexuality and health among older adults in the United States.* N Engl J Med, 2007. **357**(8): p. 762-74.
200. Harawa, N.T., et al., *Racial/ethnic and gender differences among older adults in nonmonogamous partnerships, time spent single, and human immunodeficiency virus testing.* Sex Transm Dis, 2011. **38**(12): p. 1110-7.
201. Ramaswamy, C., et al., *Human Immunodeficiency Virus Infection Newly Diagnosed at Autopsy in New York City, 2008-2012.* Open Forum Infect Dis, 2015. **2**(4): p. ofv146.
202. Lazarus, J.V. and K.K. Nielsen, *HIV and people over 50 years old in Europe.* HIV Med, 2010. **11**(7): p. 479-81.
203. Burns, F.M., et al., *Why the(y) wait? Key informant understandings of factors contributing to late presentation and poor utilization of HIV health and social care services by African migrants in Britain.* AIDS Care, 2007. **19**(1): p. 102-8.
204. Elmahdi, R., et al., *Low levels of HIV test coverage in clinical settings in the UK: a systematic review of adherence to 2008 guidelines.* Sex Transm Infect, 2014.
205. Folkhälsomyndigheten, *Hiv- och STI- prevention riktad till migranter. Available at: https://*http://www.folkhalsomyndigheten.se/pagefiles/18642/hiv-och-sti-prevention-riktad-till-migranter-webb.pdf. *Accessed 28/12/2015.* 2014.
206. Read, P., et al., *Missed opportunities for HIV testing--a costly oversight.* QJM, 2010.
207. Scognamiglio, P., et al., *The potential impact of routine testing of individuals with HIV indicator diseases in order to prevent late HIV diagnosis.* BMC Infect Dis, 2013. **13**: p. 473.
208. Joore, I.K., et al., *HIV indicator condition-guided testing to reduce the number of undiagnosed patients and prevent late presentation in a high-prevalence area: a case-control study in primary care.* Sex Transm Infect, 2015.
209. Raben, D., et al., *Auditing HIV Testing Rates across Europe: Results from the HIDES 2 Study.* PLoS One, 2015. **10**(11): p. e0140845.
210. Avery, A.K., M. Del Toro, and A. Caron, *Increases in HIV screening in primary care clinics through an electronic reminder: an interrupted time series.* BMJ Qual Saf, 2014. **23**(3): p. 250-6.
211. British HIV Association, *UK National Guidelines for HIV testing 2008. Available at:* http://www.bhiva.org/documents/Guidelines/Testing/GlinesHIVTest08.pdf. *Accessed 14/12/2015.*

212. Elmahdi, R., et al., *Low levels of HIV test coverage in clinical settings in the U.K.: a systematic review of adherence to 2008 guidelines.* Sex Transm Infect, 2014. **90**(2): p. 119-24.
213. Hill-Tout, R., I. Cormack, and A. Elgalib, *Routine HIV testing in acute medical admissions in a high prevalence area reduces morbidity and mortality of HIV: a full cycle audit.* Int J STD AIDS, 2015.
214. Lascar, M., J. Freer, and E. Phiri, *Tailoring HIV testing in a setting of late HIV diagnosis: is the tide turning?* Br J Hosp Med (Lond), 2015. **76**(10): p. 592-5.
215. Mahendran, P., et al., *Testing initiatives increase rates of HIV diagnosis in primary care and community settings: an observational single-centre cohort study.* PLoS One, 2015. **10**(4): p. e0124394.
216. ECDC., *European Centre for Disease Prevention and Control. Thematic report: Migrants. Monitoring implementation of the Dublin Declaration on Partnership to Fight HIV/AIDS in Europe and Central Asia: 2014 progress report. Stockholm: ECDC; 2015. Available at:* http://ecdc.europa.eu/en/publications/Publications/dublin-declaration-migrants-2014.pdf. *Accessed 14/1/2016.* 2015.
217. Alvarez-Del Arco, D., et al., *HIV testing policies for migrants and ethnic minorities in EU/EFTA Member States.* Eur J Public Health, 2014. **24**(1): p. 139-44.
218. Brannstrom, J., et al., *Patients unaware of their HIV infection until AIDS diagnosis in Sweden 1996-2002--a remaining problem in the highly active antiretroviral therapy era.* Int J STD AIDS, 2005. **16**(10): p. 702-6.
219. de Olalla, P.G., et al., *Epidemiological characteristics and predictors of late presentation of HIV infection in Barcelona (Spain) during the period 2001-2009.* AIDS Res Ther, 2011. **8**(1): p. 22.
220. Ekici, H., et al., *Minority drug-resistant HIV-1 variants in treatment naive East-African and Caucasian patients detected by allele-specific real-time PCR.* PLoS One, 2014. **9**(10): p. e111042.
221. Brannstrom, J., et al., *Deficiencies in the health care system contribute to a high rate of late HIV diagnosis in Sweden.* HIV Med, 2015.
222. Achhra, A.C., et al., *The Clinical Significance of CD4 Counts in Asian and Caucasian HIV-Infected Populations: Results from TAHOD and AHOD.* J Int Assoc Physicians AIDS Care (Chic), 2011. **10**(3): p. 160-70.
223. Pantazis, N., et al., *Differences in HIV natural history among African and non-African seroconverters in Europe and seroconverters in sub-Saharan Africa.* PLoS One, 2012. **7**(3): p. e32369.
224. Sasse, A., et al., *Late presentation to HIV testing is overestimated when based on the consensus definition.* HIV Med, 2015.
225. Tiittala, P.J., et al., *Achieving high acceptability of HIV testing in a population-based survey among immigrants in Finland.* Scand J Public Health, 2015. **43**(4): p. 393-8.